Life events and bipolar disorder

Kemner, Sanne

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The influence of life events on the onset and course of bipolar disorder

Sanne Mariska Kemner

Research described in this thesis was supported by grant 9120818 from the TOP Subsidy from Netherlands Organization for Scientific Research (NWO) “Determinants of the development of Bipolar disorder: two follow-up studies” and by grant 22963 from the 7th Framework program of the European Commission (Health F2-2008-222968-FP7 CP-LP MOODINFLAME)

ISBN: 978-90-367-9570-8

Cover design: Francisco Paramos
Lay-out: Persoonlijk Proefschrift, persoonlijkproefschrift.nl
Printing: Ipskamp Printing

rijksuniversiteit
groningen

Life events and bipolar disorder

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Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. E. Sterken
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

maandag 13 maart 2017 om 16.15 uur

door

Sanne Mariska Kemner

geboren op 9 november 1984
te Hoorn

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CHAPTER 1

General introduction

GENERAL INTRODUCTION

In this chapter the diagnostic criteria of bipolar disorder and risk markers associated with the onset and course of the disease are described. Subsequently, the concept of life events will be introduced and it will be explained how these can be measured, followed by a brief introduction into the twin design and genetic model fitting. Finally, the specific chapters of this thesis are introduced.

BIPOLAR DISORDER

DIAGNOSTIC CRITERIA

Bipolar disorder (BD), also known as manic depressive disorder, is characterized by episodes with severe high or low moods combined with changes in activity, sleep, energy, thinking and behaviour. The studies described in this thesis are based on the diagnostic criteria of DSM-IV (American Psychiatric Association, 1994) and not on the more recent DSM-5 (American Psychiatric Association, 2013). Therefore, the description of BD will be limited to the DSM-IV criteria.
There are four types of BD: bipolar I disorder (BD I), bipolar II disorder (BD II), cyclothymic disorder and bipolar disorder not otherwise specified (BD-NOS) (Table 1). In BD I the primary presentation consists of manic episodes which, in most cases, are alternated with depressive episodes. In BD II, the primary presentation consists of depressive episodes, alternated with hypomanic episodes. Cyclothymic disorder is a chronic state of (rapid) cycling between hypomanic and minor depressive episodes which are not reaching the diagnostic criteria for BD I or BD II. BD-NOS is characterized by bipolar features that are not meeting the criteria of any of the above mentioned disorders, e.g. very rapid alternations (days) between manic and depressive symptoms, recurrent hypomanic episodes without interference of depressive symptoms (Goodwin & Jamison, 2007; American Psychiatric Association, 1994).

CLINICAL CHARACTERISTICS OF BIPOLAR DISORDER

The prevalence of BD I and BD II is about 1-2% among adults. The lifetime prevalence of the broader bipolar spectrum ranges between 3-8.3% and is equally prevalent among the sexes (Goodwin & Jamison, 2007; Regeer, Rosso, Ten Have, Volleberg & Nolen, 2002). The age of onset of BD is best classified in three categories, namely: early onset with a mean age of 17, an intermediate onset at age 27 and a late onset with a mean age of 46 (Bellivier et al., 2011; Leboyer, Henry, Paillere-Martinot & Bellivier, 2005). The mean age of onset as found in Europe lies around 25 years (Post et al., 2008). However, BD can remain undiagnosed for many years. There is not only a delay between the first episode and the confirmed diagnosis and treatment, but also between the first symptoms and the first episode (Leverich et al., 2002).
Over 90% of BD patients experience recurrences during their lifetimes. Recurrence rates are about 40-50% over a 2 year period and 68-73% over a 4 to 5 year period (Gitlin, Swendsen, Heller & Hammen, 1995; Simhandl, Konig & Amann, 2014). The chance for recurrence is related to the number of previous episodes (Angst, Gamma, Sellaro, Lavori & Zhang, 2003; Kessing, Hansen, Andersen & Angst, 2004; Nolen et al., 2004; Simhandl et al., 2014). This is also true for the most severe episodes. After a first admission in a psychiatric hospital, 50-75% of patients have a recurrence within 4 to 5 years (Bromet et al., 2005; Leverich et al., 2001).

Table 1. Diagnostic criteria for mood episodes (American Psychiatric Association, 1994)

MANIC EPISODE

A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood for at least a week (or any duration if hospitalization is necessary)

B. During this period of mood disturbance, three (or more) of the following symptoms have persisted (four if the mood is only irritable) and have been present to a significant degree:

1. Inflated self-esteem or grandiosity

2. Decreased need for sleep

3. Pressure to keep talking

4. Flight of ideas and/or racing thoughts

5. Distractibility

6. Increased goal-directed activity or psychomotor agitation

7. Engaging in activities with potentially painful consequences

C. The symptoms do not meet criteria for a Mixed episode

D. The mood disturbance causes marked impairment in occupational functioning or social activities ore relationships with others, or necessitates hospitalization to prevent harm to self or other, or there are psychotic features

E. The symptoms are not due to the direct physiological effects of a substance or a general medical condition

HYPOMANIC EPISODE

Same as the above where episodes are not sever enough to cause marked social or occupational impairment (or necessitate hospitalization) and have no psychotic features but do represent an unequivocal change in functioning that is uncharacteristic of the person and persists for a minimum duration of 4 days

DEPRESSIVE EPISODE

A. Five (or more) of the following symptoms have been present during the same two-week period and represent a change from previous functioning: at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure

1. Depressed mood

2. Loss of interest or pleasure in activities

3. Significant change in appetite or weight

4. Insomnia or hypersomnia

5. Psychomotor agitation or retardation

6. Fatigue or loss of energy

7. Feelings of worthlessness or inappropriate guilt

8. Diminished ability to think, concentrate or make decisions

9. Recurrent thoughts of death, suicidal ideation or attempt

B. The symptoms do not meet criteria for a Mixed episode

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning

D. The symptoms are not due to the direct physiological effects of a substance or a general medical condition

E. The symptoms are not better accounted for by bereavement, i.e. after the loss of a loved one, the symptoms persist for longer than two months are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation

MIXED EPISODE

A. The criteria are met both for a Manic episode and for a Major depressive episode (except fo dureation) nearly every day during at least a 1-week period

B. The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others, or to necessitate hospitalization to prevent harm toself or others, or there are psychotic features.

C. The symptoms are not due to the direct physiological effects of a substance or a general medical condition

About 65-71% of the BD patients have psychiatric comorbid conditions. Substance use disorders (24-42%) and anxiety disorders (15-42%) are the most common comorbid disorders found in BD (Conus & McGorry, 2002). Next to this the risk for suicide among bipolar patients is about 20-30 times higher than in the general population (Pompili et al., 2013; Schaffer, Sinyor, Reis, Goldstein & Levitt, 2014). BD is also strongly related to comorbid medical conditions such as cardiovascular disease, thyroid dysfunction, diabetes and metabolic syndrome. Medical conditions may be a consequence of lifestyle, adverse effects of medication, but also point to shared etiology (Goodwin & Jamison, 2007)

(GENETIC) RISK MARKERS

The heritability of BD converges on the 60-80% range (McGuffin et al., 2003). Thus although genetic factors play an important etiological role in the disorder, the importance of environmental variables should not be neglected. Family based studies (e.g. twin studies) are crucial in research that aims to disentangle genetic from environmental sources of resemblance, later in this chapter the theory of twin studies is described in more detail. Thus far the etiology of BD is largely unresolved. BD is considered to be a multifactorial disease in which both genes and environment play a (interacting) role. A few factors associated with an increased risk for BD will be addressed.

Structural brain abnormalities

Structural neuroimaging has been used to study brain morphology in BD for over 20 years. BD is frequently associated with subtle brain abnormalities. To date the most consistent findings are increases in white matter hyper intensities and ventricular enlargement (McDonald et al., 2004; Van der Schot, 2009). In addition, there is also a large body of studies that continue to report conflicting findings such as both significantly larger and smaller volumes of the amygdala, hippocampus and thalamus among patients with bipolar disorder (Chang et al., 2005; Altshuler et al., 2000; Frazier et al., 2005; Beyer et al., 2004; Dupont et al., 1995).

The hippocampus is a brain structure that is particularly sensitive to the effects of (chronic) stressful experiences (Fuchs & Flugge, 1998; Lee, Ogle & Sapolsky, 2002; McEwen, 1999; Miller & O'Callaghan 2005; Sapolsky,1999). These stressful experiences are associated with a decreased volume of the hippocampus and with impaired hippocampal-dependent functions in patients with stress-related psychiatric syndromes, including major depressive disorder and post-traumatic stress disorder (Campbell & MacQueen, 2004; Geuze, Vermetten & Bremner, 2005; Kitayama, Vaccarino, Kutner, Weiss & Bremnder, 2005; Smith, 2005). A few studies have investigated the specific association between stressful life events and hippocampal volume in healthy subjects and found hippocampal structural deficits in relation to environmental stress. (Gianaros et al., 2007; Papagni et al., 2011; Rabl et al., 2014; Shepherd, Laurents, Matheson, Carr & Green, 2012).

In **chapter 4** we explore the relationship between life events and hippocampal volume in healthy twins.

Activation of the immune system

There is growing evidence that activation of the immune system plays an important role in the pathogenesis of BD. It was already in the early 1990's that the Macrophage T cell theory of depression was proposed (Smith, 1991), postulating an activated inflammatory response system in mood disorders. Inflammation is a part of the nonspecific immune response that takes place after any type of bodily injury or microbial invasion. Many of these reactions involve cytokines, especially interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) and IL-6, produced by dendritic cells, macrophages and other types of cells (Stertz, Magalhaes & Kapczinski 2012).
In previous studies from our group it has been reported that there is a presence of a pro-inflammatory state of circulating monocytes in a considerable proportion of not only patients with BD, but also in the offspring of bipolar parents (Padmos et al., 2008), indicating that the pro-inflammatory state of monocytes precedes the actual mood symptoms. In a subsequent twin study from our group this relation was further explored and it was concluded that common environmental factors are the main contribution to the association of the pro-inflammatory monocytes with bipolar disorder (Padmos et al., 2009).
Both major and minor stressful events can have direct adverse effects on a variety of immunological mechanisms; both animal and human studies have provided convincing evidence that these immune alterations are consequential for health (Padget & Glaser, 2003).
In **chapter 5**, we will explore the relation between BD, pro-inflammatory monocytes and stress.

Stress

Many studies have focused on the impact of stress, varying from daily hassles to traumatic events, on the development of psychiatric illnesses. In BD the focus has been on whether life events predict the timing and severity of symptoms and episodes (Johnson, 2005). Indeed, many studies have demonstrated that environmental stress plays a role in the onset and in the further course BD (Hillegers et al., 2004; Hunt, Bruse-Jones & Silverstone, 1992; Malkoff-Schwartz et al., 1998). However, the precise role of stress in the pathogenesis and course of BD remains poorly understood.
There are various models that aim to clarify the interplay between stress and psychopathology. This section contains a brief description of these models; the stress-generation hypothesis, the diathesis stress models, the kindling hypothesis and the stress buffering hypothesis.

Stress-generation hypothesis

According to the stress generation perspective, individuals are actively creating their life stressors (i.e. dependent events). Meaning that individuals vulnerable to mood disorders, when compared to those without such vulnerability, are likely to experience a higher rate of dependent events, particularly within interpersonal domains (Hammen, 1991, 2006). There is a substantial amount of support for the stress generation effect in depression (Liu & Alloy, 2010), but preliminary results from studies among both bipolar patients and bipolar offspring have failed to find support for this stress generation effect (Grandin, Alloy & Abramson, 2007; Ostiguy, Ellenbogen, Linnen, Walker & Hammen, 2009).

Diathesis stress model
Another model in which the presence of psychopathology is often explained is the stress-diathesis model (Monroe & Simons, 1991). According to this model environmental influences (stressors) trigger the onset of psychiatric disorder, because they interact with non-biological of genetic traits (diatheses).

Kindling hypothesis
An extension of the diathesis stress model is known as the kindling hypothesis, which premises that stressors (e.g. life events) are a more significant trigger in the onset of initial episodes rather than in subsequent episodes, which can at that point occur more or less spontaneously (Post, 1992). The kindling model was originally described as electrical kindling in relation to epilepsy where after many repetitions of kindled seizures 'spontaneity' occurs, i.e. seizures develop in the absence of external stimulation (Pinel, 1981). Several studies have demonstrated that a history of episodes is a significant risk factor for future recurrences in mood disorders (Judd et al., 2008; Keller, Lavori, Lewis, & Klerman, 1983; Perlis et al., 2006). However, studies to the kindling hypothesis in BD are limited and findings are inconsistent. (Bender & Alloy, 2011).

Stress buffering hypothesis
The stress-buffering model is a multifactorial model which includes possible moderators. The model posits a process in which social support is protecting persons from potentially adverse effects of stressful life events (Cohen & Willis, 1985). However, research in BD reporting on multifactorial models is scarce (Mesman, 2015).

LIFE EVENTS

Background

As described above, many studies have focused on the impact of stress on the development of psychiatric illnesses. However, the definition, operationalization and measurement of stress is not a concept that is agreed upon (Cohen, Kessler & Gordon, 1997). Cohen et al. (1997) pointed out that all studies to the impact of stress "share an interest in a process in which environmental demands tax or exceed the capacity of the organism, resulting in psychological and biological changes that may put the person at risk… [for adverse health outcomes]…". Within this definition, life events are important representations of environmental demands.
Life events can vary from regular activities occurring in daily life (daily hassles in domestic, educational or work situations) with minimal impact, to extreme situations beyond one's own control (e.g. wartime, natural disasters). In addition, life events can be both positive (e.g. a graduation) or negative (e.g. discharge from job) and can be both dependent (e.g. buying a house) and independent (e.g. natural disaster) on a person's own behaviour. This leaves us with a huge spectrum of life events that can all possibly cause a stressful experience and potentially trigger mental illness.

Measuring life events

Almost half a century ago Holmes and Rahe (1967) published a checklist of 43 events such as death of a spouse, divorce, fired at work, and sex difficulties: the Schedule

of Recent Experiences (SRE). Its purpose was to inventory "fundamentally important environmental incidents" that were found to frequently precede illness onsets. Stressful events were defined as occurrences that were likely to bring about readjustment-requiring changes in people's usual activities.
Since the publication of the SRE, a tremendous increase has occurred in the construction of such measures and in quantitative research on relations between life events and mental illness (Dohrenwend, 2006).
Checklists in the form of self-administered questionnaires or in the form of structured interviews consisting of closed questions with fixed alternative response categories have been dominant in research on the role of stressful life events in psychopathology. A large body of research on the role of life events in psychopathology that have been conducted in recent years, are making use of checklists. The behavioural genetics study of depression by Kendler et al. (1995), the nationwide epidemiological research on the comorbidity of psychiatric disorders by Kessler, Sonnega, Bromet & Nelson (1995) and the study of gene-by-environment interaction for depression by Caspi et al. (2003) are examples. The fundamental methodological puzzle in inventorying life events as risk factors for psychopathology is how to solve the problem of intracategory variability in traditional checklists. Intracategory variability is the issue that positive responses to event categories (e.g. marriage, divorce, death of a close friends) can represent very different types of actual experiences.
The dominant alternative approach in life event measurement is labour intensive and involves the collection and analysis of detailed information about the events reported. It is this narrative information about the event that makes it possible to reduce intracategory variability. If the details and context of the event are known, then the distinction between major events and minor events within a particular category become less ambiguous than if identified by a positive response to a checklist category only. For example, a positive response to 'death of a close friend' can range from 'death of a long absent, childhood friend to whom the respondent was no longer close' to 'death of a close friend, whom the respondent talked to and relied on from a day-to-day basis' depending on the respondent. This distinction can only be made if there is relevant contextual information available.
Ratings by trained judges can be made of the relevant event characteristics – such as valence, source, and magnitude – that are of interest.

LEDS

The best-known example of a narrative rating method, and the method used in all studies described in this thesis (**chapter 2-5**), is the investigator-based Bedford College Life Events and Difficulties Schedule (LEDS) developed by Brown and colleagues (Brown & Harris, 1978). Bender & Alloy (2011) confirmed that the LEDS should be considered as the gold standard of life stress measurements. Starting with its introduction over 35 years ago (Brown & Harris, 1978), this instrument was designed to deal with the problem of intracategory variability in objective scoring of checklist categories.
The LEDS is a semi-structured interview for assessing life events and long-term difficulties in adults. It collects detailed information about the event itself, the timing of its occurrence (date) and relevant contextual information for each event. Each event

is categorized into one of ten domains, consisting of education, work, reproduction, housing, money/possessions, crime/legal, health, marital/partner, other relationships and miscellaneous/death.
After conducting the interview, the interviewer writes a full report on all events. Based on the contextual information provided in this report, the threat for each event is rated via standardized rating procedures by two independent raters who have not been involved in the interviews. The threat score represents the severity of the event, ranging from mild (1) to severe (4), hereby differentiating between mild life events and more stressful life events. The contextual threat is conceptualized as: "What most people would be expected to feel about an event in a particular set of circumstances and biography, taking no account of what the respondent says either about his or her reaction or about any psychiatric or physical symptoms that followed it" (Brown & Harris, 1989).

Genes & Environment

Concurrent with the rise of the use of life events in research to environmental influence on development of psychopathology is the question whether measures of environment are influenced by genetic factors themselves (Plomin, 1986). Genetic influence on measures of environment is not as paradoxical as it seems because genetically influenced characteristics, such as cognitive abilities and personality, might affect how individuals construct their environment and how they feel about and behave towards other (Plomin, Lichtenstein, Pedersen, McClearn & Nesselroade, 1990).
Kendler and Baker (2007) reviewed 55 studies that measured the genetic influence on a wide variety of environmental factors, such as general and specific life events, parenting style, family environment, social support, peer interactions, and marital quality. Ten twin studies were identified that examined the heritability of general stressful life events (Wierzbicki, 1989; Plomin et al., 1990; Kendler, Neale, Kessler, Heath & Eaves, 1993; Billig, Hershberger, Lacono & McGue, 1996; Foley, Neale & Kendler, 1996; Thapar & McGuffin 1996; Saudino, Pedersen, Lichtenstein, McClearn & Plomin, 1997; Bolinskey, Neale, Jacobson, Prescott & Kendler, 2004; Wang, Trivedi, Treiber & Snieder, 2005). Six of these studies reported on the total SLE's (Wierzbicki 1989; Plomin et al., 1990; Kendler et al., 1993; Thapar & McGuffin 1996; Bolinskey et al., 2004; Wang et al., 2005). In five of these studies the heritabilities (i.e., the proportion of individual differences for a train in a particular population that results from inter-individual genetic differences) ranged from 24% to 47%. They concluded that every aspect of the environment that was examined, was significantly influenced by genetic factors, however the role of genetic influences was modest at most. A more recent study replicated this finding (Vinkhuyzen, van der, de Geus, Boomsma, & Posthuma, 2010), but explicitly stated that these influences were small and that the reviewed findings were often inconsistent.
The described findings suggest that what we think of as measures of the 'environment' are better described as 'external factors'. Therefore, interplay between genes and environment in the study of life events is an important facet to keep in mind when trying to disentangle how environmental factors shape individual differences in behaviour.

SAMPLES

The studies described in this thesis are from the Dutch Bipolar Offspring study (DBOS; **Chapter 3**) and the Dutch Bipolar Twin Study (DBTS; **Chapter 2, 4 and 5**). Both studies have been described in detail in previous PhD theses (DBOS; Wals, 2004; Reichart, 2005; Hillegers, 2007; Mesman, 2015. DBTS; van der Schot, 2009; Vonk, 2016; Bootsman, 2016). Below I briefly describe the samples. Demographic characteristics of both samples are presented in Table 2.

The Dutch Bipolar Offspring Study

The Dutch Bipolar Offspring Study is a prospective fixed cohort study established in 1997 with up to now a follow-up of 12 years. The main objective to initiate the Dutch Bipolar Offspring Study, was to explore the early trajectories of BD in a high risk population with the ultimate goal to be able to detect BD in an early stage and to prevent, or at least delay onset and/or diminish the severity of the illness (Reichart, 2005).
Families with at least one parent with bipolar I or II disorder having children in the age range 12-21 years old were recruited via patient associations, outpatient clinics and psychiatric hospitals.
The 140 offspring of 86 families were assessed for the baseline measurement (T1) between November 1997 and April 1999 (Wals et al., 2001). The second assessment (T2) was performed 14 months later, 132 offspring were reassessed (Reichart, Wals & Hillegers, 2007), followed by a third assessment (T3) at five year follow-up (n=129) (Hillegers et al., 2005). The fourth and most recent assessment (T4) was performed 12-years after baseline (Mesman, 2015) (Figure 1). All study assessments were approved by the Medical Ethics Committee of the University Medical Center Utrecht.

The Dutch Bipolar Twin Study

The Dutch Bipolar Twin Study is a longitudinal twin study on BD of the University Medical Center Utrecht (UMCU), The Netherlands. The main objective of this study was to examine factors related to an increased risk for bipolar disorder; i.e. obstetric complications, dermatoglypic alteration, life events, autoimmune thyroiditis with levels of thyroperoxidase antibodies, school performance and structural brain abnormalities. Twin pairs were enrolled between 2001 and 2006 for the first measurement (van der Schot, 2009; Vonk, 2016) and the second assessment was performed between 2009 and 2011 (Bootsman, 2016)(Figure 1).
Twin pairs, aged 18 to 60 years, with at least one twin suffering from either BD I or BD II were recruited via patient associations, outpatient clinics, psychiatric hospitals and Dutch media. Healthy control twins were drawn from an ongoing twin study on schizophrenia of the UMC Utrecht (Van Oel et al., 2001) and from the Netherlands Twin Register (NTR) at the VU University in Amsterdam. A total of 53 affected twin pairs took part in the study, as well as 51 control twin pairs.
Zygosity was determined by DNA fingerprinting using high polymorphic microsatellite markers to 9 to 11 in the laboratory of the Division Biomedical Genetics, University Medical Center Utrecht.

All psychiatric diagnoses were confirmed with the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon & Williams, 1996) and the Structured Interview for DSM-IV Personality (Pfohl, Blum & Zimerman, 1997). Current mood state was assessed using the Young Mania Rating Scale (YMRS – Young, Biggs, Ziegler & Meyer, 1978) and the Inventory for Depressive Symptomatology (IDS – Rush, Gllion, Basco, Jarret & Trivedi, 1996). At the time of inclusion, all patients were euthymic with an YMRS score of 4 or less and an IDS score of 12 or less. All patients were treated naturalistically.
The medical ethics review board of the UMCU approved the study and all participants gave written informed consent after full explanation of the study aims and procedures.

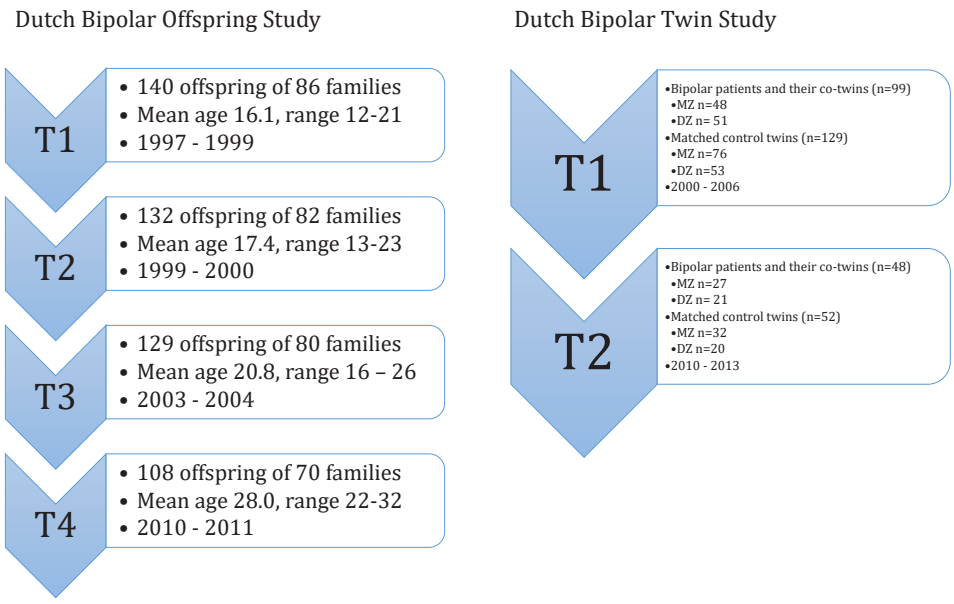

Figure 1. Study flows of the Dutch Bipolar Offspring Study and the Dutch Bipolar Twin Study

Table 2. Demographic characteristics of the Dutch Bipolar Twin Study (baseline) and the Dutch Bipolar Offspring study (T1 & T4)

	Bipolar twin pairs (n=53)		**Control twin pairs (n=51)**	
	MZ (n=24)	**MZ (n=24)**	**MZ (n=32)**	**DZ (n=19)**
Female, N (%)	34 (71)	34 (71)	40 (63)	25 (66)
Mean age, yrs (SD)	37.8 (10.6)	37.8 (10.6)	40.3 (11.5)	42.0 (7.4)
	Bipolar patients (n=66)	Non-bipolar cotwins (n=40)	Control twins (n=102)	
Female, N (%)	45 (68)	27 (68)	65 (64)	
Mean age, yrs (SD)	40.8 (10.1)	42.4 (9.9)	40.9 (10.1)	
Mean age of onset, yrs (SD) [range]	28.3 (9.7) [14 – 59]			

	Bipolar offspring T1 (n=140)	Bipolar offspring T4 (n=108)
Female, N (%)	68 (49)	50 (46)
Mean age, yrs (SD)	16.1	28.0 (2.82)
Any disorder life time, N (%)	61 (44)	78 (72)
Any mood disorder life time, N (%)	38 (27)	58 (54)
Bipolar disorder (BD I, BD II), N (%)	4 (3)	12 (11)
Age of onset first mood episode BD, yrs (SD)	14.6 (4.65)	

GENES & ENVIRONMENT

Family-based studies such as high-risk populations (offspring design) and twin studies are of indescribable value in the quest to disentangle genetic from environmental influences. The longitudinal offspring design as described in this thesis is an elegant way to explore the early trajectories of BD in a high risk population.
With this design, it is possible to detect BD in an early stage and to prevent/delay the onset and/or diminish the severity of the illness. Twin studies are crucial in research that aims to disentangle genetic from environmental sources of resemblance and are described in detail below.

Twin model

The twin design is a classic design that dates back to almost a century ago when Merriman conducted the first real twin study in 1924, to assess the genetic influence on IQ (Merriman, 1924).
Identical twins, also called monozygotic (MZ) twins derive from one fertilized egg (zygote) and are therefore genetically identical. Unlike identical twins dizygotic (DZ)

twins develop from separately fertilized eggs. They are on average 50% genetically related, equally to other siblings. If genetic factors have a significant contribution in a certain trait, MZ twins must be more similar than DZ twins. Both type of twins share many aspects of their environment (e.g., parenting style, education, socioenomic status) as they are being born and raised at the same place and time. However, both also experience unique environmental influences (e.g., unique life events, i.e. diseases, employment and peers not shared with their co-twins).
The classical twin study begins with assessing the variance of a trait in a large group of MZ and DZ twins and then estimating how much of this is due to: genetic effects (heritability), shared environment (e.g., events that happen to both twins and affect them equally,) and unique (or unshared) environment (e.g. events that are unique to one twin). These three parts are typically called A (additive genetics), C (common environment) and E (unique environment).
The basic logic of the twin design relies on the assumption that differences between MZ twins raised in the same family are due to unique environment, since they share 100% of their genes and all of the common environment. The correlation of the MZ twins provides an estimate of the proportion of the variance that can be attributed to genetic and common environmental factors. DZ twins share on average 50% of their genes and all of the common environment leading to an estimate of the correlation of DZ twins. The twin pair correlations (rMZ and rDZ), representing the resemblance of the twin pairs, offer an estimate of the relative influence to which genes or shared/unique environment determine phenotypic variation of that trait.
To study the genetic contribution of variance in a certain phenotype, the trait needs to be heritable. Heritability of a phenotype, denoted in the literature as (h^2) or alternatively as (a^2), for additive part of heritability (narrow heritability), is assumed if the MZ correlation is twice as high as the DZ correlation. The influence of common environmental factors is indicated when the correlation in DZ twins is larger than half the MZ correlation. Finally, the part of the variance where MZ twins do not resemble each other is attributable to unique environmental factors.

Genetic model fitting

Structural equation modelling (SEM) or model fitting approaches involve constructing a model that best describes the observed data. SEM is a statistical technique which tries to fit observed data to models of genetic and environmental effects. It is suitable to test whether genetic of environmental factors contribute significantly in explaining the (co)variance within or between traits (Van der Schot, 2009). SEM involves path analyses, that as defined by Ullmann (1996): 'allows examination of a set of relationships between one or more independent variables, either continuous or discrete, and one or more dependent variables'. A measured variable is a variable that cannot be observed directly and must be inferred from measured variables (also known as factors). By using path analyses, specific hypotheses about relationships between the variables are quantified by parameter estimates or path coefficients. The overall phenotypic variance is explained by using three factors: A, C and E which are latent (unobserved) variables. The factor loadings a, c, e are the parameter estimates that represent the variances due to those factors: a^2, c^2, and e^2. Parameters can be removed from the full ACE-model. For example, an ACE model is compared to an AE model. In this case the influence of common environment is excluded. The CE model excluded additive genetic influence and the E model excludes all familial

resemblance. The aim is to find the most parsimonious model that most accurately describes the observed data. This can be tested via likelihood ratio tests (LRT). This LRT statistic follows a chi-square distribution. A chi-square larger than 3.84 (1df) indicates a significant difference at alpha=0.05 and implies that the discarded effect (e.g. effect of C on a trait) cannot be left out of the model without seriously deteriorating the goodness of fit.
The liability threshold model for a disorder (e.g. BD) holds that for binary traits (presence or absence of the disorder) influenced by multiple factors of small effect, an underlying liability exists, with a threshold that divides the population into two categories for the trait. Liability is a hypothetical continuous variable that determines whether an individual will develop the disorder (Rijsdijk & Sham, 2002). In a continuum of risk it is assumed that the disorder is normally distributed within the population occurring only when a certain threshold of liability is exceeded. A person with a high value on the liability scale crossing a certain threshold would be scored 'patient' on our dichotomous variable and in all other cases considered to be healthy (discordant co-twin of patient or healthy comparison twin pairs). Since twin pairs are selected for bipolar disorder, this would result in an overestimation for the prevalence of bipolar disorder. Therefore, we fixed prevalence to 1% and heritability of bipolar disorder to 85% (Ten Have, Vollebergh, Bijl & Nolen, 2002; Regeer et al., 2004; Smoller & Finn, 2003; van der Schot 2009).
In summary, the twin design augmented by the sophisticated structural equation modelling techniques, is able to examine the extent of genetic overlap between two traits, such as a disease and a putative endophenotype (Boomsma, Busjahn & Peltonen, 2002; Hall et al., 2007). Understanding the extent of genetic overlap may be crucial, because significant genetic associations validate the proposed phenotypic measure as an endophenotype for the disorder (Lenox, Gould & Manji, 2002; Hall et al., 2007).

AIMS AND OUTLINE OF THIS THESIS

The general aim of this study is to expand our knowledge on the role of life events as potential risk factor playing a part in the onset and course of bipolar disorder.

In **chapter 2** and **3** I explore the influence of life events on onset and course of bipolar disorder.
Chapter 2 aims to clarify the role of life events on first and recurring admissions in bipolar patients including testing the role of the kindling hypothesis in this relation.
Chapter 3 aims to elucidate the interplay of life events, psychological aspects and social support on mood episode onset and recurrences among bipolar offspring

In **chapter 4** I take a side step to a healthy twin study in which we explore the influence of life events on hippocampal volume.

In **chapter 5** I aim to clarify the role of life events in the association between pro-inflammatory monocytes and bipolar disorder.

The final chapter provides a summary of all above noted chapters followed by a general discussion, clinical implications and suggestions for future research.

CHAPTER 2

The influence of life events on first and recurrent admissions in bipolar disorder

International Journal of Bipolar Disorders (2015) 3:6

S.M. Kemner, N.E.M. van Haren, F. Bootsman, M.J.C. Eijkemans,
R. Vonk, A.C. van der Schot,
W.A. Nolen and M.H.J. Hillegers

ABSTRACT

BACKGROUND

Life events play an important role in the onset and course of bipolar disorder. We will test the influence of life events on first and recurrent admissions in bipolar disorder and their interaction to test the kindling hypothesis.

METHOD

We collected information about life events and admissions across the life span in 51 bipolar patients. We constructed four models to explore the decay of life events effects on admissions. To test their interaction we used the Andersen-Gill model.

RESULTS

The relationship between life events and admissions was best described with a model in which the effects of life events gradually decayed by 25% per year. Both life event load and recurrent admissions significantly increased the risk of both first and subsequent admissions. No significant interaction between life event load and number of admissions was found.

CONCLUSIONS

Life events increase the risk of both first and recurrent admissions in bipolar disorder. We found no significant interaction between life events and admissions, but the effect of life events on admissions decreases after first admission which is in line with the kindling hypothesis.

BACKGROUND

The presence of psychopathology is often explained on the basis of stress-diathesis interactions (Monroe & Simons, 1991). The diathesis-stress model serves to explore how non-biological or genetic traits (diatheses) interact with environmental influences (stressors) to trigger the onset of psychiatric disorders (Moffitt, Caspi, & Rutter, 2005; Harris, 2001). The environmental factor most frequently studied in this context is stress, often operationalized as life events. Numerous studies have demonstrated that life events play a role in the onset and course of both unipolar depression and bipolar disorder (Bender & Alloy, 2011; Brown & Harris, 1989; Hillegers et al., 2004; Hlastala et al., 2000; Malkoff-Schwartz et al., 1998).

Methodological limitations are a major issue when interpreting and comparing studies regarding the influence of life events on the onset and course of mood disorders (Johnson, 2005). In many of these studies, data were obtained retrospectively, which complicates the reliable reporting of both life events and mood episodes due to recall bias. Moreover, regardless of the number of questions in an interview, people gradually forget life events (Paykel, 1997; Brown & Harris, 1982; Harris, 2001). Furthermore, most studies so far used the number of episodes to define course of illness whereas especially episodes longer ago are difficult to be remembered reliable, while it might be more reliable to report episodes which were associated with psychiatric admissions, as these are likely to reflect the most severe mood episodes and can often be confirmed with medical records.

The type of life event measures vary greatly between studies and pose another major obstacle in life event research. In particular, self-administered measures of stressful life events appear unreliable (Johnson, 2005). Bender & Alloy (2011) confirmed that the gold standard of life stress measurements is the Life Events and Difficulties Schedule (LEDS) (Brown & Harris, 1978). The LEDS provides the opportunity to categorize, date and rate both positive and negative life events. Furthermore, in contrast to life event questionnaires, the LEDS-interview includes both major and minor types of stress, making it more suitable for testing the kindling hypothesis where life events play a greater role in the onset of initial episodes than in subsequent later episodes, which can even occur more or less spontaneously (Post, 1992). The Kindling model was originally described as electrical kindling in relation to epilepsy where after many repetitions of kindled seizures 'spontaneity' occurs, i.e. seizures develop in the absence of external stimulation (Pinel, 1981; Wada, Sato, & Corcoran, 1974). Interestingly, several studies have demonstrated that a history of episodes is a significant risk factor for future recurrences in mood disorders (Judd et al., 2008; Keller, Lavori, Lewis, & Klerman, 1983; Perlis et al., 2006). In bipolar disorder, several studies report that after a first admission, 50-75% of patients have a recurrence within 4-5 years (Bromet et al., 2005; Leverich et al., 2001).

So far, research on the kindling hypothesis has mainly focused on unipolar depression and a majority of studies indeed found supportive evidence (Bender & Alloy, 2011). However, studies in bipolar disorder are limited and findings are inconsistent. Bender & Alloy (2011) integrated the current literature and showed that about half the studies failed to find evidence for the kindling hypothesis in bipolar disorder. Crucially, LEDS-interview based studies all failed to find such evidence (Dienes,

Hammen, Henry, Cohen, & Daley, 2006; Hammen & Gitlin, 1997; Hlastala et al., 2000; Swendsen, Hammen, Heller, & Gitlin, 1995). However, they did establish significant associations between the onset and course of bipolar disorder and life events.
In an ongoing naturalistic longitudinal twin study on bipolar disorder we obtained detailed life event information throughout the life span by using the LEDS and were able to look for possible associations with first and recurrent admissions. Our aims are (1) to assess the influence the effect of life events on first and recurrent admissions; (2) to assess the influence of prior admissions on the risk of subsequent admissions; and (3) to test the interaction between life event load and number of admissions (i.e. as indication for a kindling effect) in those twins with bipolar disorder.

METHOD

SAMPLE

We conducted a secondary analysis with three a priori questions within an ongoing study among twins (affected twin pairs n=51; healthy control twin pairs n=35) with bipolar disorder of the University Medical Center Utrecht (UMCU), The Netherlands. Of this cohort all 51 twins with bipolar disorder (bipolar I disorder, n=37; bipolar II disorder, n=14) were included in the current study. The design of the study and the recruitment of the bipolar twin pairs has been described in detail elsewhere (Van der Schot et al., 2009; Vonk, van der Schot, Kahn, Nolen, & Drexhage, 2007). All participants were enrolled between 2001 and 2006. There were no restrictions on duration or stage of illness for inclusion in the study and all patients were treated naturalistically.
Demographic information is displayed in table 1. All diagnoses were confirmed with the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon, & Williams, 1996) and the Structured Interview for DSM-IV Personality (Pfohl, Blum, & Zimmerman, 1997). Hospitalizations were confirmed through available medical records. Current mood state was assessed using the Young Mania Rating Scale (YMRS; Young, Biggs, Ziegler, & Meyer, 1978) and the Inventory for Depressive Symptomatology (IDS; Rush, Gullion, Basco, Jarrett, & Trivedi, 1996). At the time of the study, all patients were euthymic with a YMRS score of 4 or less and an IDS score of 12 or less.
The study was approved by the medical ethics review board of the University Medical Center Utrecht and all participants gave written informed consent after full explanation of the study aims and procedures.

Table 1. demographics

	Total	Bipolar Disorder Type 1	Bipolar Disorder Type 2
N	51	37	14
Female / Male (n)	33 / 18	25 / 12	8 / 6
Age LEDS-interview, M (SD)	40.49 (9.52)	39.51 (8.71)	43.07 (11.33)
Age onset first bipolar episode, M (SD)	28.20 (9.16)	26.19 (6.94)	33.50 (12.16)
Age onset first symptoms (all), M (SD)	26.08 (8.85)	25.27 (6.82)	28.21 (12.86)
Age onset treatment, M (SD)	27.72 (9.03)	26.41 (7.71)	31.46 (11.59)
Comorbid disorder (1, 2 or 3) n(%)	11 (22%)	8 (22%)	3 (21%)
Psychotic symptoms lifetime, n(%)	26 (51%)	24 (65%)	2 (14%)
Hospitalized group			
Hospitalized patients, n(%)	35 (69%)	31 (84%)	4 (29%)
Number of admissions M (SD),	3.06 (2.45)	2.93 (2.11)	4.00 (4.69)
Age first admission, M (SD)	27.91 (7.86)	26.61 (6.4)	38 (11.83)
Type of episode at first admission (n);			
Mania	15	15	0
Depression	12	9	3
Psychosis	6	6	0
Other	2	1	1

LIFE EVENT MEASURES

All subjects included in the current study were interviewed with the investigator-based Bedford College Life Events and Difficulties Schedule (LEDS; Brown & Harris, 1978; Brown & Harris, 1989). The LEDS is a semi-structured interview for assessing life events and long-term difficulties in adults. It collects detailed information about the event itself, the timing of its occurrence (date) and relevant contextual information for each event. Each event is categorized into one of ten domains, consisting of; education, work, reproduction, housing, money/possessions, crime/legal, health, martial/partner, other relationships, miscellaneous/death. Based on the contextual information, the threat for each event is rated via standardized rating procedures. The threat score represents the severity of the event, ranging from mild (1) to severe (4), hereby differentiating between mild life events and more stressful life events. The contextual threat is conceptualized as: "What most people would be expected to feel about an event in a particular set of circumstances and biography, taking no account of what the respondent says either about his or her reaction or about any psychiatric or physical symptoms that followed it" (Brown & Harris, 1989). Several studies have supported the reliability (e.g. interrater) and validity (e.g. multiple informant) of the LEDS in adults exhibiting a variety of psychiatric symptoms (Brown & Harris, 1978; Brown & Harris, 1989; Ormel, Oldehinkel, & Brilman, 2001).

Only events occurring from the age of 5 years were included. All severe events were defined by the extent they were related to the bipolar disorder and to what extent they were dependent of the respondents own behaviour. To determine relatedness to the disorder each severe event was rated on a three point scale; 1) not related to psychopathology; 2) possibly related to psychopathology or; 3) clearly related to psychopathology. Only events with score 1 were included for further analyses. To determine if life events occurred independent of will or influence of the respondents own behaviour each severe event was rated on a 7 point scale; 1) completely independent; 2) nearly independent; 3) possible influence, however, very unlikely; 4) physical illness; 5) cooperation or agreement with external situation; 6) likely neglect or carelessness; and 7) intentional choice. Events rating 1 to 5 were included in further analyses. Each life event was dated per year. Age was then calculated for each event.
All interviewers and raters were trained by MH, who was trained by G.W. Brown and T.O. Harris, who developed the LEDS. The interviews were conducted at the participants home or at the UMCU. Events were rated by two independent raters who had not been involved in the interviews. A panel consisting of the four raters (including SK and MH) reached consensus on the events that raised rating problems.

STATISTICAL ANALYSIS

Life event load

Life event load represents the sum of the threat scores of the life events occurring in each year. We calculated three different life event load measures; (1) cumulative load (CL), i.e. the life event load at a particular point in time (year Y) calculated as the sum of the life event load in year Y and all preceding years, (2) cumulative load excluding events possibly or clearly related to the bipolar disorder (CL-NoBP), and (3) cumulative load including only independent events, thus excluding events possibly or clearly dependent of the respondents own behaviour (CL-I).
Next, the life event load before the first, or since the last admission was calculated. After each admission, life event load was reset to zero and was calculated as described above. The cumulative life event load in the year preceding the admission was used for analysis.

Decay model

Previous studies showed a decay effect, implying that the presumed effect of life events diminish over time, e.g. the death of a close relative that occurred three or four years before admission has less impact compared to the same event one year before admission (Hillegers et al., 2004). We will investigate which decay model statistically fits the data best. To explore the degree to which the effect of life events diminishes over time, a time-specific life event load variable was calculated for every year and subjected to an exponential decay function. We tested four models; in model I we tested the purely cumulative effect, in models II to IV the decay function implied a 25%, 50%, and 75% loss of effect per year, respectively. The decay-function yielding the best model fit (-2x log likelihood) will be used for all further analysis.

Andersen-Gill Model

The Andersen-Gill model (A-G model), an extension of the standard Cox proportional hazards model for recurrent events, accommodates censored data and time dependent covariates (Fleming & Harrington, 1991; Therneau & Grambsch, 2000). Data for the A-G model are structured such that for each individual intervals at risk are defined by variables describing the start and end times of each year of age. An event variable is coded as "1" for admission and "0" for no admission.

The A-G approach follows the usual assumption of the Cox model that the hazard or risk ratio is proportional over time and more specifically, that the risk of being admitted is unaffected by earlier admissions. Time dependent covariates, such as the cumulative load of life events or the number of previous admissions, may be used to relax the latter assumption. The hazard ratio represents the proportionate change in the 'admission' rate due to a unit change in the respective covariate, in this case the cumulative life event load.

Andersen-Gill Model; interaction effect

The presence of an interaction effect will be tested by integrating an interaction function in the A-G model, testing the effect of the interaction between the number of admissions and the cumulative load between the admissions in the best-fitted decay-model; also known as a kindling effect (Post, 1992).

RESULTS

The general characteristics of our sample are shown in Table 1. At least one admission had occurred for 35 of the 51 bipolar patients, with a maximum of eleven admissions in two patients. Figure 1 and Table 2 display the number and polarity for all admissions.

Table 2. Number of admissions

Total number of admissions	N (total = 51)
0	16
1	8
2	10
3	7
4	5
5	1
6	1
7	1
11	2

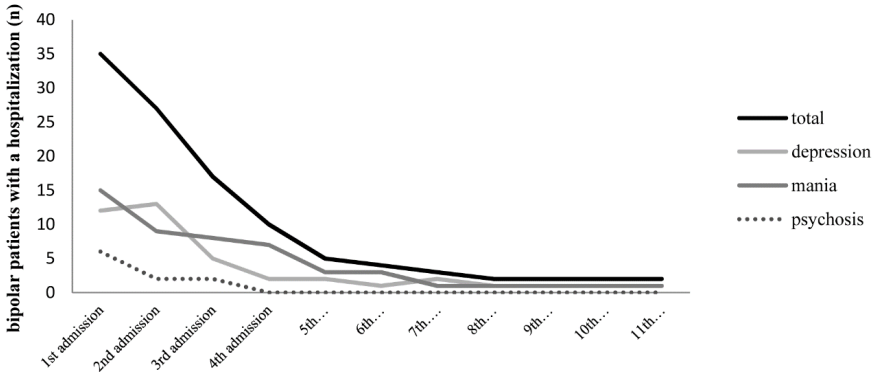

Figure 1. Number and polarity of admissions

Influence of life events effect on first and recurrent admissions

The relationship between life event load and admission (irrespective of the number of admissions) is depicted in Table 3. The exponentiated linear coefficients from an A-G model are interpreted as risk ratios relating the magnitude of a covariate (or multiple covariates) to admission. Positive coefficients indicate increased hazard for admission ('1' vs '0'). Figure 2 illustrates the cumulative life event load over time and the cumulative life event load between the admissions.

Independent of the model employed (cumulative, 25%, 50% or 75% decay), the life event load was significantly associated with an increased risk of hospitalization per unit life event load. Adjustment for age and gender did not change the life event risk ratios. According to the log-likelihood, indicating the quality of fit, the decay model in which the life event load accumulates and at the same time decreases with a function of 25% with every subsequent year (model II) was most in agreement with the observed data. Therefore, all further analyses will be done under model II. Figure 3 illustrates the cumulative life event load over time and between admissions, both under the 25% decay model.

Table 4 displays the results of the A-G model with the three different types of load between the admissions; cumulative load (CL), cumulative load excluding events that were related to the bipolar disorder (CL-NoBP) and cumulative load included only independent events (CL-I).

All coefficients for both life event load and number of admissions are positive and significant. Positive effect of all types of life event load, indicates that the risk of getting admitted grows with an increasing life event load. This effect is independent of the type of life event load.

The A-G model with life time cumulative load and number of admissions, shows a positive and significant risk ratio for both the life time load (coef=.0985, SE=.0166, $p<.001$) and number of admissions (coef=.4597, SE=.0892, $p<.001$), indicating that in addition to the cumulative load between the admissions, the life time cumulative load also contributes to the risk of getting admitted.

Table 3. Relative risk of admission using four models of events effect decay

Model	Coefficient	Exp Coefficient[1]	Log-likelihood of fitted model	*p*
Cumulative	.024	1.024	-308.5503	<.001
25% decay	.134	1.143	-293.5533[2]	<.001
50% decay	.240	1.272	-298.4862	<.001
75% decay	.452	1.572	-306.3194	<.001

[1]exponentiated linear coefficients
[2]lowest absolute log-likelihood of fitted model

Table 4. Influence of different types of cumulative load between admissions and number of admissions on the chance of getting admitted (all under 25% decay model)

Type of Cumulative Load between admissions [1]	coefficient	exp coef[2]	SE (coef)	robust SE[3]	z	p
CL	.086	1.09	.019	.021	4.17	<.001
Number of admissions	.560	1.75	.064	.093	6.03	<.001
CL-I	.093	1.10	.023	.021	4.35	<.001
Number of admissions	.577	1.78	.066	.099	5.81	<.001
CL-NoBP	.085	1.09	.027	.024	3.59	<.001
Number of admissions	.603	1.83	.069	.111	5.43	<.001
Interaction effect						
CL	.071	1.07	.027	.026	2.79	<.001
Number of admissions	.513	1.67	.091	.115	4.47	<.001
CL x Number of admissions	.006	1.01	.009	.008	0.75	.45
CL-I	.081	1.08	.030	.023	3.49	<.001
Number of admissions	.537	1.71	.091	.118	4.56	<.001
CL-I x Number of admissions	.006	1.01	.009	.010	0.58	.57
CL-NoBP	.053	1.05	.035	.024	2.25	<.05
Number of admissions	.510	1.67	.093	.125	4.07	<.001
CL-NoBP x Number of admissions	.018	1.02	.012	.015	1.23	.22

[1] CL; cumulative load including all events
CL-I ; cumulative load including only independent events
CL-NoBP; cumulative load excluding events related to the disorder
[2] Exponentiated coefficients, representing the hazard ratio
[3] Robust SE (standard error), corrected for the dependency of multiple times to event within the same subject

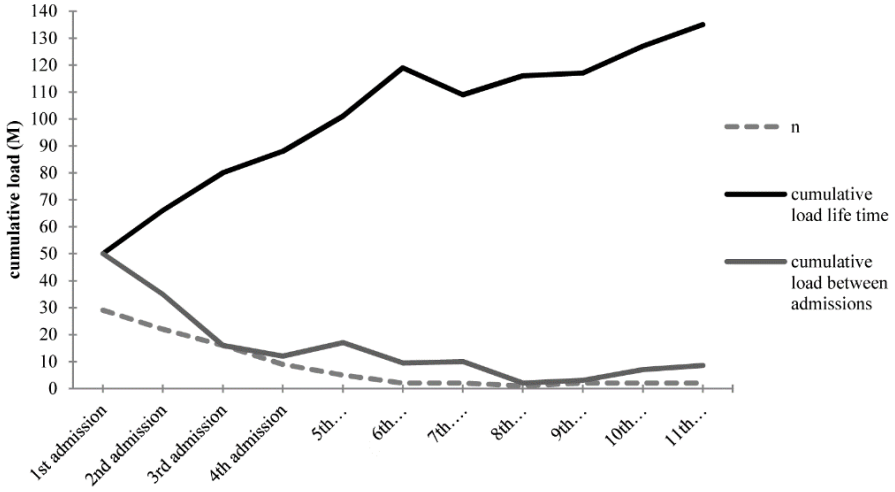

Figure 2. Course of cumulative load

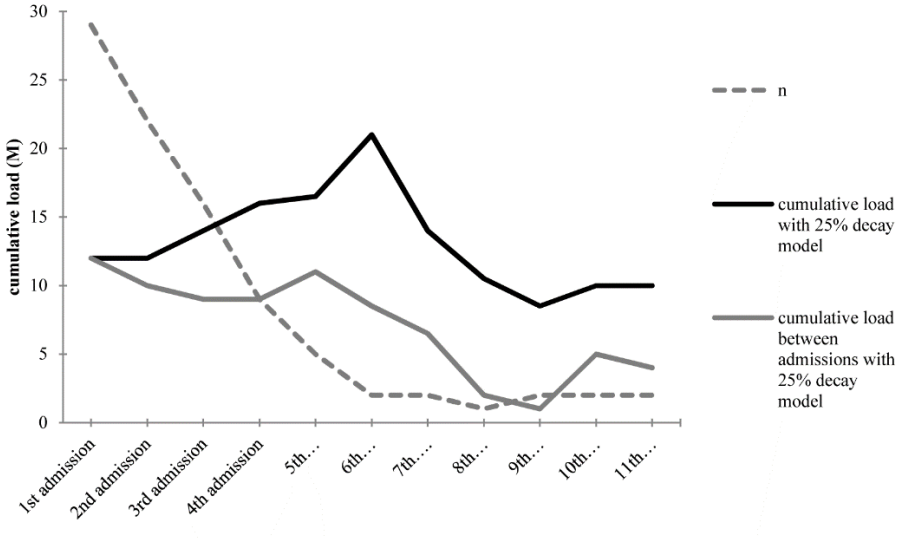

Figure 3. Course of cumulative load under the 25%-decay model

Effect of number of previous admissions on admissions

The positive and significant coefficient of number of admissions on the risk of getting admitted, implies an increase in the chance of getting admitted with each subsequent admission.

Interaction between life event load and number of admissions

The interaction effect between the cumulative load between admissions in model II (with 25% decay) and number of admissions did not reach significance, indicating that the effect of cumulative load on the risk of admission does not change with

subsequent admissions. However, the influence of life events on first admissions is higher compared with the influence of life events after on re-admissions, suggesting a shift in the effect of life events between first and subsequent admissions.
Results did not change when excluding the concordant co-twins from the sample. Also, age, age of onset of the first bipolar episode, age of first admission nor gender affected any of the above findings.

DISCUSSION

Our main finding is that an increased life event load, taking into account the number and threat of life events, impacts both first and recurrent admissions in bipolar patients. This has also been found in previous studies (Bender & Alloy, 2011; Hunt, Bruce-Jones, & Silverstone, 1992; Kessing, Andersen, & Mortensen, 1998; Kessing, Agerbo, & Mortensen, 2004), but it was hypothesized that this might be due to life events occurring as a consequence of the disease (Kessing et al., 2004). We now extended these previous findings by showing that the effect of life events on admissions did not change when events related to the disorder were excluded from the analyses. This suggests that the effect of life events is independent of life events occurring in relation to the disorder. We consider this robust influence of life events on first and recurrent admissions an important finding, as exposure and responses to life events are potentially modifiable. A better understanding of how they impact the risk of being admitted may yield specific strategies for prevention and early intervention.
Our next finding was that the effect of number of prior admissions on the risk of getting admitted was positive and significant, demonstrating that the risk increases with each admission. Several studies reported that after a first admission for bipolar disorder, 50-75% of patients relapse within 4-5 years (Bromet et al., 2005; Leverich et al., 2001). Our findings indicate that the risk of readmission increases as a function of the number of previous admissions. Given our finding that the risk of getting admitted is independent of events that are related to the disorder, such as admissions, the association between number of previous admissions and increased risk of readmission might be interpreted as an indicator for illness severity. Moreover, this finding also suggests a possible kindling effect; a previous admission could trigger the next admission.
Finally, we found no significant interaction between life event load and the number of prior admissions on the risk to be readmitted, suggesting that the effect of life event load does not decrease as a function of subsequent admissions. However, we did find a stronger effect of life events on first compared to subsequent admissions which does suggest a possible kindling effect. In this respect it should however be realized that the kindling effect has mostly been found after the occurrence of 5 to 7 episodes (Kendler, Thornton, & Gardner, 2000; Kendler & Gardner, 2001; Slavich, Monroe, & Gotlib, 2011) while we only looked at admissions and the average number of admissions lies between 3 and 4 in our sample. Previous studies using the LEDS in bipolar patients (Dienes et al., 2006; Swendsen et al., 1995; Hammen & Gitlin, 1997) looking at episodes rather than admissions did not find evidence for either

presence or absence of a kindling effect. This in contrast with findings in unipolar depression, which might be explained by the more complex course of contrasting mood episodes (i.e. mania and depression) in bipolar disorder as compared with unipolar depression (only depression). Bipolar episodes can be manic, hypomanic, depressive or mixed and it is possible that the influence of life events differs across these various episodes.

The effect of life events on admissions was best described by model II in which the influence of life events steadily accumulates (as one gets older more life events occur), but at the same time gradually decays with 25% per year as time goes by (an event that has occurred years ago will no longer have the same impact as when it just happened). This decay model is in accordance with previous findings from our group in a sample of offspring of parents with bipolar disorder (Hillegers et al., 2004). The decay model of 25% best explained the influence of stressful life events on the onset of mood disorders when compared to the purely cumulative model or models with 50% or 75% decay per year. The underlying mechanisms that cause this decay are not known; a possible explanation lies in the interaction of life stress with coping strategies and temperament. Coping responses influence the association between stress and the onset of mood episodes. Temperamental traits influence individual coping styles and modify the impact of stressful life events on mood episode onset (Compas, Connor-Smith, & Jaser, 2004).

There are several limitations that need to be taken into account when interpreting our findings. First, methodological limitations are a major issue when interpreting and comparing studies regarding the influence of life events on the onset and course of mood disorders (Johnson, 2005). In many of these studies, information on life events was obtained retrospectively with queries or (semi-) structured interviews, which complicates the reliable reporting due to recall bias. Regardless of the number of queries in an interview, people gradually forget life events (Paykel, 1997; Brown & Harris, 1982; Harris, 2001). The average participant in our sample had to report life events over a time span of 35 years. One could question the reliability of the LEDS when it is used retrospectively to collect lifetime life event data. Most studies restrict the reporting of life events to a 12-month period. However, the LEDS is probably more reliable compared to (retrospective) checklist inventories (Hillegers et al., 2004; Ormel et al., 2001), as the LEDS minimizes recall bias; information is actively obtained in a very structured interview by detailed questions in ten domains. Furthermore, there is evidence that recall bias is more pronounced for minor events, suggesting that major life changes are under less influence of recall bias (Funch & Marshall, 1984).

Secondly, more than one admission could occur per year. It is not clear to what extent this influenced our results, since admissions occurring within 3-6 months after the first admission are associated with more subclinical affective symptoms and therefore could be due to the same bipolar episode (Bromet et al., 2005). Unfortunately, the data on life events was dated per year and did not allow us to conduct the analysis in more detail.

We made no distinction between admissions due to mania, depression or psychosis. However, as can be seen in Figure 1, the polarity of the admissions is equally divided across the number of admissions for manic and depressive episodes.

Our sample is drawn from a longitudinal twin study. Having participants in the sample that, to a large extent, share their genes and environment might influence the study results. However, excluding the bipolar cotwins (n=8) resulting in only one twin per pair in the analysis (n=43) did not change our findings.
Finally, although most analysis yielded significant results, we have a small sample size consisting of patients with bipolar I as well as bipolar II disorders. So far, most studies limit their sample to bipolar type I (Bender & Alloy, 2011). The small sample size did not allow us to compare the two subtypes.

CONCLUSION

Life events, taking into account the number and threat of life events, appeared to have an impact on both first and recurrent admissions in bipolar patients and this effect appeared not be dependent on events related to the illness. In addition, the number of prior admissions was positively related to the risk of getting readmitted. Finally, we did not find an interaction between life events and admissions on the risk for readmission, although the effect of life events was stronger on first admissions compared to readmissions, which suggests a possible kindling effect.

CHAPTER 3

The role of life events and psychological factors in the onset of first and recurrent mood episodes in bipolar offspring: results from the Dutch Bipolar Offspring Study

Psychological Medicine 2015 April 13; x(x): 1-11

Sanne M. Kemner*, Esther Mesman*, Willem A. Nolen,
René M. J. C. Eijckemans and Manon H.J. Hillegers
* Authors contributed equally to this
manuscript and share first authorship.

ABSTRACT

BACKGROUND

Life events are an established risk factor for the onset and recurrence of unipolar and bipolar mood episodes, especially in the presence of genetic vulnerability. The dynamic interplay between life events and psychological context, however, is less studied. In this study, we investigated the impact of life events on the onset and recurrence of mood episodes in bipolar offspring, as well as the effects of temperament, coping, and parenting style on this association.

METHODS

Bipolar offspring (n=108) were followed longitudinally from adolescence to adulthood. Mood disorders were assessed with: the Kiddie Schedule of Affective Disorders and Schizophrenia – Present and Lifetime Version or the Structured Clinical Interview for DSM-IV Axis-I disorders; life events with the Life Events and Difficulties Schedule; and psychological measures using the Utrecht Coping List, Temperament and Character Inventory and short-EMBU (memories and upbringing instrument). Anderson Gill Models (an extension of the cox-proportional hazard model) were utilized.

RESULTS

Life events were associated with an increased risk for first and, although less pronounced, subsequent mood episodes. There was a large confounding effect for the number of previous mood episodes; findings suggest a possible kindling effect. Passive coping style increased the risk of mood episode onset and recurrent episodes, but also altered the effect of life events on mood disorders. Harm avoidance temperament was associated with mood episode recurrence.

CONCLUSIONS

Life events are especially a risk factor in the onset of mood disorders, though less so in recurrent episodes. Psychological features (passive coping and harm avoidant temperament) contribute to the risk of an episode occurring, and also have a moderating effect on the association between life events and mood episodes. These findings create potential early intervention strategies for bipolar offspring.

INTRODUCTION

Bipolar disorder (BD) is characterised by episodes of depression and (hypo)mania, alternated with periods of euthymia. Typically, BD presents with a (mild) depressive episode, whereas the first (hypo)manic episode appears years later (Duffy, Alda, Hajek, & Grof, 2009; Mesman, Nolen, Reichart, Wals, & Hillegers, 2013). On average, this typical early course leads to a 10 year diagnostic delay (Drancourt et al., 2013; Altamura et al., 2010; Suppes et al., 2001). Presently, the most reliable predictor for BD remains a positive family history for BD (Gottesman, Laursen, Bertelsen, & Mortensen, 2010; Craddock & Jones, 1999). Several studies have consistently shown that children of patients with BD (bipolar offspring) have an increased risk for bipolar spectrum disorders, as well as (recurrent) unipolar mood disorders (Lapalme, Hodgins, & LaRoche, 1997; Duffy et al., 2011; Mesman et al., 2013).

Apart from a positive family history, stressful life events are associated with the onset of first as well as subsequent mood episodes in BD (Bender & Alloy, 2011; Brown & Harris, 1989a; Hlastala et al., 2000; Malkoff-Schwartz et al., 1998; Koenders et al., 2014; Johnson, 2005). However, the understanding of the precise role of stressful life events in the pathogenesis and the course of BD, remains quite poor. There is also evidence that life events are particularly influential with regard to the first number of mood episodes, yet become less so as subsequent episodes emerge; also known as the 'kindling hypothesis' (Bender & Alloy, 2011; Monroe & Harkness, 2005; Post, 1992). However, the results of studies like these are inconsistent. Evidence for the kindling hypothesis would emphasise the importance of studying the role of life events in populations at risk of developing BD prior to the onset of the first episode, such as bipolar offspring.

Presently, only a few studies have investigated the role of life events in bipolar offspring. Overall, these studies identified an increased number of life events and/or more severe life events in bipolar offspring (Duffy et al., 2007; Hillegers et al., 2004; Ostiguy et al., 2009; Wals et al., 2005; Petti et al., 2004). Two studies reported on life events in the Dutch Bipolar Offspring Study. Wals *et al*. (2005) found an increased number of life events preceding the year of mood episode onset, but this effect faded when controlling for prodromal mood symptoms in that same year. However, this study only took into account life events in the year preceding the onset of the first mood episode. While a single life event may only have a moderate effect on mood susceptibility, it is likely that especially the accumulation of events gradually increase mood susceptibility (Kessing, Agerbo, & Mortensen, 2004). It is, therefore, particularly interesting to follow the course of life events across the life cycle in relation to the onset of mood episodes. The second Dutch Bipolar Offspring Study found an association between life events and the onset of the first mood episode in 140 bipolar offspring age 5 up to 16, while the adverse effects of life events gradually subsided by 25% per year (Hillegers et al., 2004).

Studies in unipolar depression have found that the interplay between psychosocial factors and life events is also important. Life events are not equally stressful to everyone and their effect depends on several factors, such as temperament, coping, cognitive styles and social support; the so called stress-buffering hypothesis (Cohen & Wills, 1985; Compas, Connor-Smith, Saltzman, Thomsen, & Wadsworth, 2001;

Compas, Connor-Smith, & Jaser, 2004; Swendsen, Hammen, Heller, & Gitlin, 1995). Two studies found a relation between maladaptive cognitive styles and increased reactivity to life events in bipolar patients (Alloy, Reilly-Harrington, Fresco, Whitehouse, & Zechmeister, 1999; Reilly-Harrington, Alloy, Fresco, & Whitehouse, 1999; Swendsen et al., 1995). One study in bipolar offspring (Duffy et al., 2007) reported an increased number of recent life events in bipolar offspring with psychopathology, while emotionality was positively correlated with recent life events and psychopathology. Moreover, emotionality contributed to the risk of psychopathology, whereas life events only functioned as a mediator. However, a limitation of this study was that life events were not assessed longitudinally, and both life events and temperament measures were only assessed after the onset of the illness.
This study investigates the association of stressful life events on the onset of first and recurrent mood episodes, in the same Dutch Bipolar Offspring cohort as reported above (Wals et al., 2005; Hillegers et al., 2004), now followed up 12 years to a mean age of 28 years. Furthermore, we investigated the effects of psychological factors such as temperament, coping and parental rearing styles on this association.

METHOD

SAMPLE

All data are derived from the Dutch Bipolar Offspring Study, a longitudinal fixed cohort study established in 1997 (Wals et al., 2001; Reichart et al., 2004; Hillegers et al., 2005; Mesman et al., 2013). A detailed description of the study design and recruitment procedure has been described elsewhere (Wals et al., 2001). In short, 140 offspring (ages 12-21) from 86 families with one bipolar parent (74% bipolar I; 26% bipolar II) were recruited from 1997-1999 and followed for a period of 12 years. A family was only included if all offspring within the age range 12-21 agreed to participate. Exclusion criteria were a severe physical illness or handicap or an IQ below 70. Participants were recruited through the Dutch patient association (62 families; 102 children) and through outpatient clinics in nine psychiatric hospitals (24 families; 38 children). Bipolar offspring were assessed at baseline and at one-, five-, and 12-year follow-up (Wals et al., 2001; Reichart et al., 2004; Hillegers et al., 2005; Mesman et al., 2013). In total, 108 (77%) subjects were followed for the full 12-years. The study was approved by the Medical Ethical Review Committee of the University Medical Center Utrecht. Written informed consent was obtained from both offspring and parents after a complete description of the study.

INSTRUMENTS

Mood disorders

Offspring were psychiatrically evaluated at each assessment; at baseline and during the one-year follow-up DSM-IV diagnoses were obtained through direct interviews with both child and parent(s), using the *Kiddie Schedule of Affective Disorders and Schizophrenia Present and Lifetime Version* (K-SADS-PL) (Kaufman et al., 1997). From the five-year follow-up onwards, the K-SADS-PL was replaced by the *Structured Clinical Interview for DSM-IV Axis I Disorders* (SCID-I) (First, Spitzer, Gibbon,

& Williams, 1997). Lifetime DSM-IV diagnoses are based on all four interviews. For a detailed overview of the psychopathology after 12-year follow-up, see Mesman *et al.* (2013). In the current study, the presence of mood disorders was extrapolated by identifying a lifetime history of DSM-IV major depressive disorder, dysthymia, cyclothymia, bipolar I- or II disorder, depression NOS or adjustment disorder with depressed mood. For depression NOS only 'minor depressive disorder' and 'recurrent brief depressive disorder' were included. Moreover, because of the perceived uncertainty of the bipolar disorder not otherwise specified (BD-NOS) diagnosis (Goodwin & Jamison, 2007), BD-NOS was not specifically assessed. Recurrent mood disorder was defined as any consecutive mood episode/disorder after the first episode (e.g. depression NOS and subsequent major depressive disorder). For all diagnoses, both onset-age and episode(s) duration were documented.

Stressful life events

Life events were assessed with *the investigator-based Bedford College Life Events and Difficulties Schedule* (LEDS), a semi-structured interview assessing life events and long-term difficulties (Brown & Harris, 1978; Brown & Harris, 1989), adjusted for adolescents (Monck & Dobbs, 1985). The present study focussed solely on life events. The LEDS collects detailed information about the event itself, the timing of its occurrence (date) and relevant contextual information for each event. Based on the contextual information, the threat for each event is rated via standardized rating procedures. The threat score represents the severity of the event, ranging from mild (1) to severe (4). The contextual threat is conceptualized as: "What most people would be expected to feel about an event in a particular set of circumstances and biography, taking no account of what the respondent says either about his or her reaction or about any psychiatric or physical symptoms that followed it" (Brown & Harris, 1989). Severe events could be negative as well as positive, for example: moving to another country can be a very positive, but at the same a time stressful, life event. No distinction was made between positive and negative life events. Because of the retrospective nature of the data, only severe events (threat score 3 or 4) that had occurred after age 4 were included for analyses. For all severe life events we defined whether the event was related to the respondents' psychopathology and whether the event was dependent on the respondents own behaviour. Several studies support the reliability and validity of the LEDS in adults exhibiting a variety of psychiatric symptoms (Brown & Harris, 1978; Brown & Harris, 1989; Ormel, Oldehinkel, & Brilman, 2001). The LEDS interviews were administered at each assessment. The interviews covered life events from early childhood (after age 4) up to baseline assessment and for the interim periods at the follow-up assessments. Life events were rated on a yearly basis. All interviewers and raters were trained by MH, who herself was trained by Brown and Harris who developed the LEDS. The events were rated from written transcripts by two independent raters who had not been involved in the interview, and were unaware of the respondent's psychiatric status. A panel consisting of five raters, including SK, EM and MH, reached consensus on the events that raised rating problems.

Temperament

Temperament was examined using the Dutch adaption of the short version of the *Temperament and Character Inventory* (TCI) (105-items) (Cloninger, przybeck, Svrakic, & Wetzel, 1994; Duijsens & Spinhoven, 2001; Duijsens, Spinhoven, Verschuur, & Eurelings-Bontekoe, 1999; Cloninger, 1994). The TCI is based on the neurobiological model of Cloninger (Cloninger, 1994), measuring four temperament dimensions (*novelty seeking, harm avoidance, reward dependence and persistence*) and three character dimensions (*self-directness, cooperativeness and self-transcendence*). Temperament reflects the developmentally stable personality components from infancy through adulthood, and character is thought of as the part of the personality that gradually matures throughout life (Cloninger, 1994). To maintain a certain stable personality measurement, only temperament scales were used for further study. Each dimension contains 15 items with a true/false-scoring. The Dutch adaption of the TCI shows modest to good reliability in terms of internal consistency with Cronbach ranging from .69 (reward dependence) up to 0 .89 (harm avoidance) and good test-retest reliability (.71 to .90)(Duijsens & Spinhoven, 2001).

Coping

Coping was assessed with the *Utrecht Coping List* (UCL), a self-report questionnaire of 47-items that measures 7 coping styles (Scheurs, van de Willege, Tellegen, & Brosschot, 1993). The items describe possible reactions to problem situations or unpleasant events, and are answered on a 4-point response scale ranging from rarely to never. The seven coping styles include active tackling (6 items), i.e. the individual actively approaches the problem situation and is goal-oriented; palliative response (8 items), i.e. distraction, engaging in other activities and trying to relax; avoidance and passive expectancy (8 items), i.e. avoidance of the problem situation and waiting to see what happens; seeking social support (6 items), i.e. sharing feelings of discomfort and seeking support and understanding from others; passive reacting (7 items), i.e. being completely overwhelmed by the situation, pessimistic and withdrawn; *expression of emotion* (3 items), i.e. venting emotions of discomfort such as anger and irritation; and *reassuring thoughts* (5 items), i.e. realizing worse things can happen and positive reframing of the situation. The UCL has moderate to good internal consistency with Cronbach α ranging from 0.64 to 0.82 and reasonable test-retest reliability 0.52 to 0.78 (Scheurs et al., 1993).

Parental rearing

The respondents' subjective experience of parental rearing (both father and mother) was assessed by use of the short-EMBU (Swedish acronym for Egna Minnen Beträffande Uppfostran [My memories of upbringing]; s-EMBU) at the 5-years follow-up (Arrindell, Emmelkamp, Brilman, & Monsma, 1983; Arrindell et al., 2001; Perris, Jacobsson, Lindstrom, von, & Perris, 1980). The s-EMBU is a 23-item with 4-point Likert type response scale and examines three parenting rearing styles: *emotional warmth* (6 items), *protection* (9 items), and *rejection* (7 items). *Emotional warmth* refers to affectionate, stimulating and praising behaviour in the parent; *protection* refers to fear and anxiety for the subject's safety, and intrusive and overinvolved behaviour of the parent; and *rejection* refers to punitive behaviour, shaming, favouring a sibling, rejection through criticism, rejection of the subject and abusive behaviour. All were found

to have good internal reliability with Cronbach $\alpha > 0.70$ (Arrindell et al., 2001). The correlation of parenting styles of mothers and fathers were moderate to high in this study (emotional warmth, $r = 0.58$, protection, $r = 0.68$ and rejection $r = 0.72$). For further analyses these scores were combined to a mean total score per parenting style. Temperament, coping and subjective parental rearing styles were all assessed during the 5-year follow-up of the study.

DATA ANALYSIS

Time dependent life event load

In order to study the impact of life events at the onset of first and subsequent episodes, a time dependent life event load (LEL) for each year of follow-up was calculated, representing the sum of all severe life events (threat scores 3 & 4). The cumulative life event load (CLEL) at a particular point in time (year Y) was calculated as the sum of the life event load in year Y and all preceding years. For the impact of life events on the onset of a first mood episode, we calculated a CLEL for the year before its onset. For the impact of *recurrent* episodes the CLEL load started at zero after each episode. Subsequently, the CLEL in the year preceding recurrence was used for analysis. Overall, we calculated three different types of life event measures: cumulative load (CLEL); cumulative load excluding events related to psychopathology of the respondent (CLEL-NoPsy); and cumulative load including only independent events (CLEL-Ind). Taking into account a possible decay effect of life events over time, a time-specific life event load was subjected to an exponential decay function (Wainwright & Surtees, 2002). We tested four models: model I tested a purely cumulative effect of life events (=CLEL); model II the decay function implied a 25% loss of CLEL per year; model III the decay function implied a 50% loss of CLEL per year; and model IV the decay function implied a 75% loss of CLEL per year. The decay-function that yielded the best model fit (-2* log likelihood) was subsequently used for all further analysis.

Statistical analyses

The impact of life events and the onset and recurrence of mood disorders was studied using an extension of the standard cox-proportional hazard model for recurrent events, the Andersen-Gill model (A-G model). The A-G model accommodates censored data and time dependent covariates (Fleming & Harrington, 1991; Therneau & Grambsch, 2000). Data for the A-G model was structured in such a way that each individual risk interval was defined by variables describing the start and end times of each year of age. An event variable was coded “1” for episode and “0” for no episode. Time from age 5 to first mood episode was used or, when no episode occurred, the time until the last interview was used to test the influence of life events on mood episode onset. To test the impact of life events on recurrent episodes, the time until the last interview was included regardless of whether one or more episodes occurred. The A-G approach follows the usual assumption of the Cox model, whereby the hazard or risk ratio is proportional over time, and more specifically, that the risk of developing a mood episode is unaffected by earlier episodes. Time dependent covariates, such as the CLEL or the number of previous episodes, may be used to relax the latter assumption. The hazard ratio represents the proportionate change

in the 'episode' rate due to a unit change in the respective covariate, in this case the CLEL. Subsequently, temperament, coping styles, subjective parental rearing style, plus the number of episodes were added as covariates in the A-G model. Our aim was to examine whether these variables affected the risk of mood episode onset and/or recurrence, and the impact of life events. A moderating effect was considered present if inclusion of these variables substantially (by at least 10%) changed the coefficient for life event load. If a significant moderating effect of any of these three psychological factors was present, the interaction between those factors and all other psychological factor was tested. This was realised by incorporating an interaction function into the A-G model. Finally, the presence of a kindling effect was tested by the interaction between the number of previous episodes and the CLEL between episodes. Analyses were performed under the statistical programming platform R (R Development Core Team, 2008).

RESULTS

The general characteristics of the study population are shown in table 1. In total, 68 (54%) of the 140 offspring were diagnosed with a lifetime mood disorder, of which 38 had a history of one or more recurrent episodes, including 16 (24%) with bipolar spectrum disorder. Of the offspring with a recurrent mood disorder, the median number of recurrent episodes was 4 (range 2-36). Descriptives of CLEL, temperament-, coping- and subjective parenting styles are shown in table 1. In order to take into account a temporal decay effect of life events impact, life event data was fitted to four different models with decay function 0%, 25%, 50% and 75% (model I-IV respectively) (table 2). According to the log-likelihood, the decay function of 75% (model IV) was in most agreement with the observed data for the first mood episode, and for recurrent mood episodes the decay function of 50% (model III). Since the difference between the log-likelihoods of the fitted models was minimal, all further analyses were performed under model III. Figure 1A/B displays the difference in course of CLEL and the CLEL according to model III for mood affected offspring versus unaffected offspring per year.

Table 1. Descriptive characteristics

	Bipolar offspring	
	n = 140	
Mean age at first assessment, years (range)	16.1	(12-21)
	N	%
Gender, boys	72	51
Mood affected offspring	68	49
First mood episode:		
Major depressive episode	21	
Dysthymia	10	
Cyclothymia	3	
Depression NOS	31	
Adjustment disorder, depressive type	3	

Table 1. Descriptive characteristics - *Continued*

Mean age of onset of first mood episode (range)	16.8	(7-28)	
Offspring with recurrent episodes	36	26	
Median # of episodes (range)	4	(2-36)	
Offspring with a current mood episode at one of the 4 interviews	38	27	
Non mood disorders[a]	26	19	
No disorder[b]	46	33	
	Mean	**(S.D.)**	**Min - Max**
Life event load			
CLEL	36.2	(20.4)	6 - 134
CLEL-Ind	25.6	(14.5)	0 - 86
CLEL-NoPsy	23.1	(14.9)	3 - 107
	Mean	**(S.D.)**	**Min - Max**
Temperament			
Novelty seeking	8.3	(2.6)	2 - 14
Harm avoidance	5.8	(3.2)	0 - 14
Reward dependence	9.2	(2.6)	1 - 14
Persistence	7.7	(3.0)	1 - 15
Coping style			
Active tackling	17.8	(3.2)	9 - 24
Palliative response	17.2	(3.8)	8 - 26
Avoidance and passive expectancy	15.8	(2.9)	9 - 22
Seeking social support	14.3	(3.2)	6 - 24
Passive reacting	10.6	(2.5)	7 - 18
Expression of emotion	6.2	(1.4)	3 - 10
Reassuring thoughts	11.7	(2.9)	6 -19
Parental rearing style			
Emotional warmth	17.6	(2.9)	10.5 - 23
Protection	16.9	(3.2)	10 - 24.5
Rejection	8.6	(1.8)	7 - 18

CLEL, cumulative life event load including all events under model III (50% decay model); CLEL-Ind, cumulative life event load including only independent events; CLEL-NoPsy, cumulative life event load excluding events related to psychopathology.

[a]Including all subjects without a lifetime mood disorder or dropping out from the study without developing a mood disorder, but with other non-mood pathology. Including anxiety, attention-deficit hyperactivity disorder, disruptive behaviour, substance abuse, enuresis, encopresis, pervasive developmental disorder, tic, obsessive—compulsive disorder and eating disorders.

[b]Offspring without a lifetime DSM-IV axis I disorder before the end of/leaving the study.

Table 2. Relative risk of an episode using four models of decay onset and lifetime

	Life event load onset			Life event load lifetime		
Model	Coefficient	Exp coef	-2 log-likelihood	Coefficient	Exp coef	-2 log-likelihood
I (cumulative)	0.005	1.01	-287.1	0.020	1.02	-836.9
II (25% decay)	0.049	1.05	-286.0	0.087	1.09	-825.0
III (50% decay)	0.127	1.14	-284.9	0.181	1.20	-824.7[a]
IV (75% decay)	0.348	1.42	-283.9[a]	0.415	1.51	-826.0

Exp coef, Exponentiated linear coefficients and 95% confidence interval
[a] Lowest absolute log-likelihood of fitted model

FIRST MOOD EPISODE ONSET

As shown in Table 3, the CLEL up to the first mood episode was associated with a positive coefficient of 0.127 (HR = 1.14) indicating the increased relative risk for mood episode onset per increase per unit of CLEL. Next, we looked at the different types of life events, namely: CLEL including only independent events (CLEL-Ind), excluding events related to psychopathology of the respondent (CLEL-NoPsy). The coefficient of CLEL-Ind was 0.177 (HR = 1.19) and was also positively associated with mood episode onset per increase in unit CLEL. A coefficient of 0.104 (HR = 1.11) was found for CL-NoPsy, and did not reach significance, suggesting that life events triggering mood disorder onset also includes events already associated with previous non-mood disorders.

Looking at psychological features and social support, only harm avoidant temperament, passive reacting coping style, and a rejecting parenting style were significantly associated with first mood episode onset (table 4). Yet, only passive reacting coping style altered the coefficient of CLEL by more than 10% from 0.127 (HR = 1.14) to 0.196 (HR = 1.22), suggesting that having more passive reacting coping style features, enhances the risk of mood episode onset, whereas the impact of CLEL decreases, suggesting a moderating effect. All other coefficients for psychosocial factors did not reach significance (for an overview of all temperament, coping and parental rearing styles see Table S1, available online).

Table 3. Influence of different types of life event load under model III (50% decay) on mood episodes

Type of Cumulative Load[a]	coefficient	exp coef[a]	SE (coef)	robust SE[b]	z	*p*
Mood episode onset						
CLEL	0.127	1.14	0.052	0.056	2.26	<0.05
CLEL-Ind	0.177	1.19	0.082	0.081	2.19	<0.05
CLEL-NoPsy	0.104	1.11	0.098	0.090	1.15	0.25
Recurrent mood episodes						
CLEL	0.112	1.12	0.022	0.031	3.67	<0.001
CLEL-Ind	0.106	1.11	0.026	0.0298	3.54	<0.001
CLEL-NoPsy	0.123	1.13	0.0323	0.039	3.16	<0.05
Recurrent mood episodes and previous episodes						
CLEL	0.078	1.08	0.024	0.036	2.18	<0.05
Previous episodes[c]	0.181	1.20	0.014	0.029	6.31	<0.001
Recurrent mood episodes; interaction with previous episodes						
CLEL	0.105	1.11	0.027	0.031	3.38	<0.001
Previous episodes[c]	0.204	1.22	0.018	0.035	5.81	<0.001
CLEL x Previous episodes[d]	-0.007	.993	0.004	0.003	-1.91	0.056

S.E., Standard error; CLEL, cumulative life event load including all events under model III (50% decay model); CLEL-Ind, cumulative life event load including only independent events; CLEL-NoPsy, cumulative life event load excluding events related to psychopathology.

[a] Exponentiated coefficients, representing the hazard ratio

[b] Robust SE (standard error), corrected for the dependency of multiple times to event within the same subject

[c] Expresses the relative risk per each episode

[d] Interaction term

Table 4. Influence of life event load, psychological and social factors on mood episode onset

		coefficient	exp coef[a]	*p*
Mood episode onset				
Baseline model	CLEL	0.127	1.14	<0.05
Temperament	CLEL	0.127	1.14	<0.05
	Harm avoidance	.099	1.10	<0.05
Coping	CLEL	0.196*	1.22	<0.05
	Passive reacting	0.209	1.23	<0.001
Parental rearing	CLEL	0.124	1.13	<0.05
	Rejection	0.252	1.29	<0.001
Recurrent mood episodes				
Baseline model	CLEL	0.078	1.08	<0.05
	Previous episodes[b]	0.181	1.20	<0.001
Temperament	CLEL	0.059**	1.06	0.08
	Previous episodes[b]	0.167	1.18	<0.001
	Harm avoidance	0.084	1.09	<0.05
Coping	CLEL	0.057**	1.06	0.12
	Previous episodes[b]	0.169	1.18	<0.001
	Passive reacting	0.162	1.18	<0.001
Parental rearing	CLEL	0.069**	1.07	<0.05
	Previous episodes[b]	0.171	1.19	<0.001
	Rejection	0.151	1.16	<0.001
	CLEL	0.066**	1.07	0.056
	Previous episodes[b]	0.182	1.20	<.001
	Protection	0.073	1.08	<0.05

CLEL, Cumulative life event load including all severe events under model III (50% decay model);previous episodes, number of previous episodes.

[a] Exponentiated coefficients, representing the hazard ratio

[b] Expresses the relative risk per each episode.

*main effect is significant & coefficient for life events changes >10%; <0.114 or >0.140

**main effect is significant &coefficient for life events changes >10%; <0.0702 or >0.0858

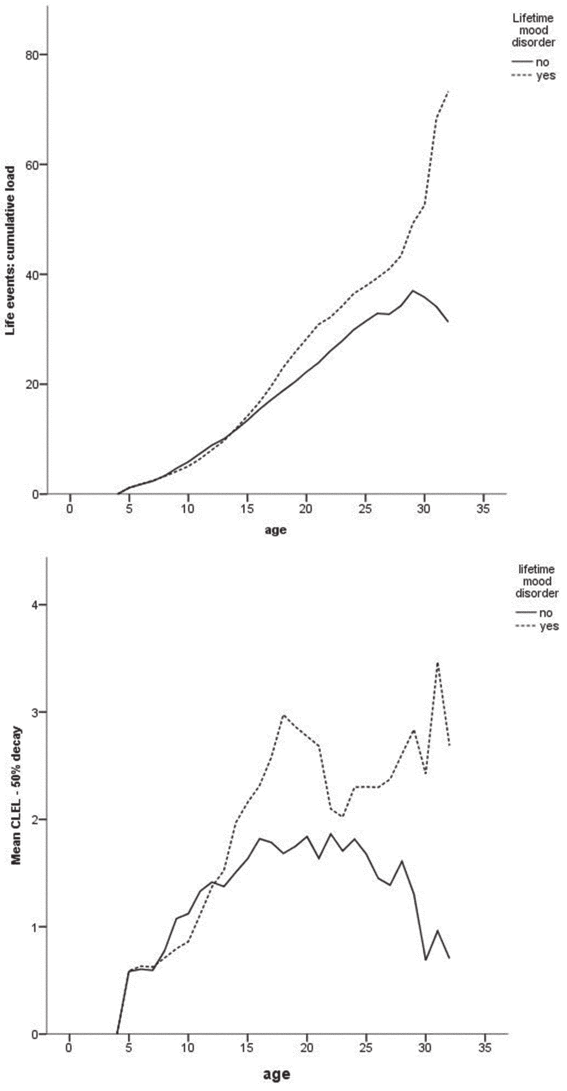

Figure 1. (A) Course of cumulative life event load by age (years) for mood affected and unaffected offspring. (B) Course of cumulative life event load by age (years) according to decay model III for mood affected and unaffected offspring (bottom)

RECURRENT MOOD EPISODES

For recurrent mood episodes a coefficient of 0.112 (HR = 1.12, p<0.001) for CLEL on the risk of mood episodes was found. Also, CLEL-Ind and CLEL-NoPsy showed significant and positive coefficients (CLEL-Ind 0.106, HR = 1.11, p < 0.001 and CLEL-NoPsy 0.123, HR = 1.13, p = <0.05), indicating that all types of life event load are associated with an increased risk of recurrent episodes. In Table 3 provides the results of the final A-G model of LEL, as modelled for recurrent episodes. Adding the number of previous episodes in the model decreased the coefficient for life events by more than

10% with a positive and significant coefficient for previous episodes, indicating that the number of previous episodes is a significant contributor to the risk of recurrent episodes. In addition, a greater number of previous episodes is associated with a lower CLEL preceding episode onset; since the amount of time between the episodes decreases, so does the CLEL preceding the episode. The interaction term for CLEL and number of prior episodes effect did not reach significance, indicating that the effect of CLEL on the risk of recurrence does not change with subsequent episodes, as suggested by the kindling hypothesis. However, significance reached trend level and the influence of life events on first episode was higher compared with the influence of life events after the first episode; suggesting a shift in the effect of life events between first and subsequent episodes and thus a possible kindling effect.
Apart from the CLEL and number of previous episodes, harm avoidant temperament, passive reacting coping style and a rejecting and protective parenting style were significantly associated with the risk for recurrent episodes and altered the impact of CLEL (table 4). Including these factors into the model, in addition to the number of previous episodes, lowers the impact of life events on mood episode recurrence. The change of the coefficient of life events was largest for harm avoidant temperament and passive reactive coping style. Furthermore, for these two factors the coefficient for life events not only decreased, but also became non-significant. The coefficients of these two factors were positive, indicating that the presence of a more pronounced harm avoidant temperament and/or passive reactive coping style increases the risk of recurrent episodes. Adding these four psychosocial factors decreased the coefficient of life events, though not reduced to zero, indicating that, although not significant, life events are still present as a risk factor for recurrent episodes. For an overview of the effects of the other psychological and social factors, see Table S2.

DISCUSSION

To our knowledge, this is the first long-term follow-up study investigating the impact of life events and psychological variables on the onset and course of psychopathology in bipolar offspring. In this study, bipolar offspring were followed for 12 years, from early adolescence (N=140) to adulthood (N=108). In total, 68 offspring developed a mood disorder, of which 36 a recurrent mood disorder. The results illustrate that the effect of life events is especially a risk factor in the early stage of mood disorders, and that this effect is enhanced by passive reactive coping styles in bipolar offspring. After the first episode, the number of previous episodes became an important predictor for new episodes, with a shift in the effect of life events between first and subsequent episodes; supporting the kindling hypothesis. Moreover, psychological factors, such as harm avoidant temperament and passive reactive coping style, increase the risk of subsequent episodes.
That life events are associated with mood episode onset in bipolar offspring confirms findings in other offspring studies (Duffy et al., 2007; Ostiguy et al., 2009; Petti et al., 2004). The impact of life events was the strongest with regard to the first episode, becoming less so with recurrent episodes. Although we found no interaction between previous episodes and CLEL, the impact of life events becoming less

evident corresponds with a possible kindling effect. Thus far, the kindling effect has consistently been reported in unipolar depression, but irregularly in BD (Dienes, Hammen, Henry, Cohen, & Daley, 2006; Swendsen, Hammen, Heller, & Gitlin, 1995; Hammen & Gitlin, 1997).

Previous research has suggested that especially dependent life events (e.g. life events related to the individual's own behaviour) are important in light of mood episode onset (Hammen, 1991). In line with others (Reilly-Harrington et al., 1999; Grandin, Alloy, & Abramson, 2007; Ostiguy et al., 2009), we found that differentiating between dependent and independent life events did not change our findings; suggesting that no specific type of life event contributes to increased mood liability, but rather the full range of severe life events.

Regarding the psychological factors we found that a more passive reactive coping style, defined as being overwhelmed by situations, pessimism, and withdrawal, was associated with mood episode onset. This finding is in line with previous research, where especially the disengaging coping styles are related to an increased risk of internalizing symptoms (Compas et al., 2001). More interestingly, adding passive reactive coping style enhanced the association between life events and mood episode onset; suggesting a moderating effect for passive reactive coping style, thus supporting the stress-buffering theory in this population (Cohen & Wills, 1985; Compas et al., 2001; Compas et al., 2004; Swendsen et al., 1995a). For recurrent episodes, especially harm avoidant temperament and passive reactive coping style were important predictors: while the association between life events and recurrent episodes was less pronounced, it did not become redundant. Parental rearing styles were not found to have an additional effect on the association between life events and mood episode onset. In summary, our study suggests that life events play an important role in the early stages of mood episode onset, yet psychological factors such as a negative temperament and/or passive reactive coping style may be of more significance for recurrent mood disorder susceptibility.

Strengths in our study are that life events were recorded over the life course within relatively short retrospective time frames, limiting the effect of recall bias. Furthermore, a natural decay effect of life events was taken into account, aiming to model the natural processes of life events. Moreover, life events were recorded using the LEDS of Brown and Harris; the suggested golden standard in life event research (Dohrenwend, 2006). Nevertheless, the findings of this study should be interpreted in the light of several limitations. The first limitation of the study is that temperament, coping, and parental rearing styles were administered at the third assessment (mean age 21). Although the present study shows that harm avoidant temperament and passive reacting coping style are associated with the susceptibility for mood episodes in a high risk population, the direction of the impact of these factors remains partly intangible. Temperament has been suggested to be relatively stable over time and across situations, but there are also suggestions that temperament can be changed by previous mood episodes (e.g. Compas et al., 2004). Secondly, due to the small sample size of subjects with BD, it was not possible to run separate analyses for depressive or hypomanic episodes. Thirdly, the present study concerns a high risk population, limiting generalisation for other populations. However, studies have shown that the impact of life events is especially significant within the context

of familial risk for mood disorders (e.g. Kessler, 1997; Zimmermann et al., 2008). More studies are needed to further disentangle the interplay of life events, psychological factors, impact of social support and mood susceptibility. These studies could benefit by incorporating not only early assessment of psychological factors, in order to determine directions of associations, but also considering more diverse populations, and including healthy controls and larger sample sizes. In the meantime, our findings suggest that early intervention on stress-reduction in terms enhancement of coping skills through training, cognitive behavioural therapy or EMDR in case of more severe trauma, might potentially prove beneficial in preventing onset and recurrences op mood episodes in high risk populations. In conclusion, our study shows that both life events and psychological factors play an important role in bipolar offspring regarding their susceptibility to develop first as well as recurrent mood episodes. These findings provide potential for early intervention.

SUPPLEMENTAL MATERIAL

Table S1. Confounding effects of psychological and social factors on life events and mood episode onset

		coefficient	exp coef[a]	p
Baseline	CL	0.127	1.14	<0.05
Temperament				
	CL	0.124	1.13	<0.05
	Novelty seeking	0.071	1.07	0.24
	CL	0.127	1.14	<0.05
	Harm avoidance	0.099	1.10	<0.05
	CL	0.133	1.14	<0.05
	Reward dependence	0.071	1.07	0.20
	CL	0.126	1.13	<0.05
	Persistence	0.033	1.03	0.46
Coping				
	CL	0.215	1.24	<0.05
	Active tackling	-0.065	.93	0.09
	CL	0.215	1.24	<0.05
	Palliative response	0.051	1.05	0.15
	CL	0.221	1.24	<0.05
	Avoidance and passive expectancy	-0.013	.986	0.76
	CL	0.209	1.23	<0.05
	Seeking social support	0.041	1.04	0.28
	CL	0.196*	1.22	<0.05
	Passive reacting	0.209	1.23	<0.001
	CL	0.215	1.24	<0.05
	Expression of emotion	0.13	1.14	0.16
	CL	0.222	1.25	<0.05
	Reassuring thoughts	-0.037	.964	0.40
Parental rearing				
	CL	0.124	1.13	<0.05
	Rejection	0.252	1.29	<0.001
	CL	0.126	1.13	<0.05
	Emotional warmth	-0.121	.886	<0.05
	CL	0.121	1.13	<0.05
	Protection	0.021	1.02	0.60

CL = cumulative life event load including all severe events under model III (50% decay model).

[a] Exponentiated coefficients, represent the hazard ratio

* main effect is significant & coefficient for life events changes >10%; <.114 or >.140

Table S2. Confounding effects of psychological and social factors on life events and mood episodes recurrence

		coefficient	exp coef[a]	*p*
Baseline model	CL	0.078	1.08	<.05
	Previous episodes	0.181	1.20	<.001
Temperament				
	CL	0.077	1.08	<.05
	Previous episodes	0.195	1.22	<.001
	Novelty seeking	0.060	1.06	.30
	CL	0.059*	1.06	.08
	Previous episodes	0.167	1.18	<.001
	Harm avoidance	0.084	1.09	<.05
	CL	0.078	1.08	<.05
	Previous episodes	0.178	1.20	<.001
	Reward dependence	0.028	1.03	.60
	CL	0.081	1.08	<.05
	Previous episodes	0.183	1.20	<.001
	Persistence	0.059	1.06	.11
Coping				
	CL	0.085	1.08	<.05
	Previous episodes	0.181	1.19	<.001
	Active tackling	- 0.027	.97	.43
	CL	0.082	1.09	<.05
	Previous episodes	0.176	1.19	<.001
	Palliative response	0.022	1.02	.47
	CL	0.085	1.09	<.05
	Previous episodes	0.177	1.19	<.001
	Avoidance and passive expectancy	-0.006	.99	.86
	CL	0.081	1.08	<.05
	Previous episodes	0.177	1.19	<.001
	Seeking social support	0.044	1.05	.31
	CL	0.057*	1.06	.12
	Previous episodes	0.169	1.18	<.001
	Passive reacting	0.162	1.18	<.001
	CL	0.079	1.08	<.05
	Previous episodes	0.179	1.20	<.001
	Expression of emotion	0.153	1.17	.11
	CL	0.086	1.09	<.05
	Previous episodes	0.178	1.20	<.001
	Reassuring thoughts	-0.02	.98	.63
Parental rearing				
	CL	0.069*	1.07	<.05
	Previous episodes	0.171	1.19	<.001
	Rejection	0.151	1.16	<.001
	CL	0.071	1.07	.056
	Previous episodes	0.185	1.20	<.001
	Emotional warmth	-0.062	.93	.16
	CL	0.066*	1.07	.056
	Previous episodes	0.182	1.20	<.001
	Protection	0.073	1.08	<.05

CL, cumulative load including all severe events under the 50% decay model between episodes; previous episodes, number of previous episoded

[a] Exponentiated coefficients, represent the hazard ratio

* Main effect is significant & coefficient for life events changes >10%; <0.0702 or >0.0858

CHAPTER 4

The association between hippocampal volume and life events in healthy twins

Hippocampus. 2016 Aug; 26(8): 1

F. Bootsman*, S.M. Kemner*, M.H.J. Hillegers, R.M. Brouwer, R. Vonk, A.C. van der Schot, H.E. Hulshoff Pol, W.A. Nolen, R.S. Kahn and N.E.M. van Haren
* Authors contributed equally to this manuscript and share first authorship

ABSTRACT

Hippocampal volume deficits have been linked to life stress. However, the degree to which genes and environment influence the association between hippocampal volume and life events is largely unknown. In total, 123 healthy twins from monozygotic and dizygotic twin pairs underwent magnetic resonance imaging (MRI), and 57 healthy twins were interviewed with the Life Events and Difficulties Schedule (LEDS), with an overlap of 54 twins undergoing both MRI and the life events interview. Hippocampal volumes were segmented with Freesurfer software. Data were analyzed with OpenMx software.Smaller hippocampal volume was associated with higher severe life event load (r_{ph} = -0.39), where shared environmental factors influencing both measures fully explained the association. Hippocampal volume was not associated with total or mild life event load. Hippocampal volume showed high heritability (h^2: 69%) whereas life event measures were influenced by shared (c^2) and unique (e^2) environmental factors only (range, c^2:40%-64%, e^2: 36%-60%). Our results suggest that shared environmental factors influence the relationship between smaller hippocampal volume and severe (but not mild) stress. This indicates that particularly severe life events that are shared between twins are associated with smaller hippocampal volume. Furthermore, we suggest to distinguish between mild and severe life events in life event research.

INTRODUCTION

The hippocampus is a highly plastic brain structure (Gu, Janoschka, & Ge, 2013; Spalding et al., 2013), implicated in memory and emotional processing (Aldhafeeri, Mackenzie, Kay, Alghamdi & Sluming., 2012; Phillips, Ladouceur & Drevets., 2008; Bird & Burgess, 2008; Milner, Squire, & Kandel, 1998). Importantly, it provides negative feedback signaling to the hypothalamus during the hypothalamic-pituitary-adrenal (HPA) activated response to environmental stress, a process which results in secretion of glucocorticoids that are vital to short-term survival (Jankord &Herman, 2008). However, chronic stress has been linked to disruption of hippocampal negative feedback signaling (Conrad, 2006) and to glucocorticoid toxicity in the hippocampus itself, which, as a result, may show structural atrophy and diminished neurogenesis (Brown et al., 2004; Gianaros et al., 2007; Mirescu & Gould, 2006; Sapolsky, Uno, Rebert & Finch, 1990; McEwen, 2007).

Only a few studies have examined hippocampal structural deficits in relation to environmental stress by specifically investigating the association between stressful life events and hippocampal volume in healthy subjects, suggesting evidence for a negative association between them (Gianaros et al., 2007; Papagni et al., 2011; Rabl et al., 2014; Shepherd, Laurens, Matheson, Carr, & Green, 2012). Remarkably, there is a large variability in stress-assessment strategies when determining the number and impact of life events, as well as differences in demographics (e.g. age) of studied groups. For example, one recent study in young adults reported that smaller hippocampal volume was predicted by total number of self-reported stressful life events, but only in interaction with specific genetic variants (COMTVal158Met, BDNFVal66Met and 5-HTTLPR) (Rabl et al., 2014). In contrast, a study in postmenopausal women showed that smaller hippocampal grey matter volume was predicted by higher stress scores, where subjects were required only to indicate the perceived degree of control, predictability and overload of life events on a 4-item self-report questionnaire without describing the event itself (Gianaros et al., 2007). Yet another, longitudinal study reported that hippocampal volume loss over time was associated with higher number of stressful life events in a sample of young adults (Papagni et al., 2011).

Importantly, little is known about the genetic and environmental contributions to the association between hippocampal volume and life events. In this respect, twin studies provide a suitable approach to determine the relative influence of genes, and shared and unique environmental factors on measured traits as well as on the association between traits. Therefore, in this healthy twin study, we examined (1) whether hippocampal volume was associated with life events as assessed with the elaborate semi-structured Bedford College Life Events and Difficulties Schedule (LEDS; Brown & Harris, 1978; Brown & Harris, 1989), and (2) the relative contribution of genes and (shared and unique) environmental factors to hippocampal volume, life events, and the association between them.

MATERIALS AND METHODS

SUBJECTS

A total of 123 healthy twins from monozygotic (MZ) and dizygotic (DZ) twin pairs (MZ: 35 twin pairs and 4 subjects from incomplete pairs; DZ: 22 twin pairs and 5 subjects from incomplete pairs) underwent magnetic resonance imaging. Furthermore, a group 57 healthy twins (MZ: 15 twin pairs; DZ: 13 twin pairs and 1 twin from incomplete pairs) were interviewed on life events with the semi-structured Life Events and Difficulties Schedule (LEDS). Here, 54 twins underwent both MRI scanning and the LEDS interview. Due to the limited availability and motivation of some of the subjects in the MRI sample, the LEDS interview could not be conducted in all subjects that underwent MRI. However, as there were no differences in inclusion or exclusion criteria between the LEDS sample and the MRI sample, we feel confident that the twins whose life events were assessed are representative of the larger sample.

Healthy twins were taken from previously described twin samples (Baaré et al., 2001; Brans et al., 2008; van der Schot et al., 2009). Of the total group of 126 twins that either underwent MRI or were interviewed on life events, 47 healthy control twins were originally recruited by van der Schot et al. (2009). Of the remainder of 79 twins, 18 twins were taken from the cohort that was described by Baaré et al. (2001) and 61 twins were taken from the cohort that was included by Brans et al. (2008). These twins were originally recruited from the (healthy) twin sample of the department of Psychiatry of the University Medical Center Utrecht and the Netherlands Twin Registry (Boomsma, 1998; Baaré et al., 2001). Subjects were between 18 and 60 years of age (see table 1 for demographic information). MZ and DZ twin pairs were matched for age and parental education.

As many of these twins served as control subjects in studies with patients with schizophrenia and bipolar disorder, they had no history of axis I psychiatric disorder or axis II personality disorder according to *DSM-IV* criteria. This was confirmed with the Structured Clinical Interview for DSM-IV (SCID) (First, Spitzer, Gibbon & Williams, 1996), and the Structured Interviews for DSM-IV Personality (SIDP and SCID-II; First et al., 1996; Pfohl, Blum & Zimmerman, 1997). Moreover, they had no history of severe medical illness. None of the subjects had drug or alcohol dependency in the last six months prior to inclusion. Furthermore, the twins had no first-degree relatives with a history of a major axis I psychiatric disorder (DSM-IV) such as bipolar disorder, schizophrenia, psychotic disorder, major mood disorder, anxiety disorder or substance-related disorder. Family histories of the twins were obtained via the Family Interview Genetic Studies (FIGS; Nurnberger et al., 1994), performed with both twins. Zygosity was determined in the laboratory of the Division Biomedical Genetics, University Medical Center Utrecht (UMCU) with DNA fingerprinting using high polymorphic microsatellite markers 9 to 11. The medical ethics review board of the UMCU approved the study and all participants gave written informed consent after full explanation of the study aims and procedures.

Table 1. Demographic characteristics and descriptive statistics of hippocampal volume and life event measures for all subjects.

	Total group[a] (n=126)	MZ (n=75)	DZ (n=51)	F	df	p
Gender, m/f	54/72	30/45	24/27			
Age, y, mean (sd)	38.44 (8.96)	38.64 (9.92)	38.14 (7.41)	0.10	1, 124	0.76
Parental educ., y, mean (sd)	11.49 (3.36)	11.41 (3.33)	11.61 (3.44)	0.10	1, 124	0.75
Education,y, mean (sd)	13.56 (2.52)	13.73 (2.66)	13.29 (2.3)	0.92	1, 124	0.34
Hippocampal volume (in ml), mean (sd)[b]	8.52 (0.74)	8.59 (0.75)	8.41 (0.72)	1.61	1, 121	0.21
Total life event load, mean (sd)[c]	80.74 (21.55)	73.4 (21.23)	88.89 (19.15)	8.30	1, 55	0.01
Mild life events, load, mean (sd)[c]	66.09 (16.84)	60.97 (16.64)	71.78 (15.43)	6.42	1, 55	0.01
Severe life events, load, mean (sd)[c]	14.65 (8.42)	12.43 (7.8)	17.11 (8.54)	4.67	1, 55	0.04

Abbreviations: MZ, Monozygotic; DZ, Dizygotic
n= number of individuals
[a] Total number of subjects who were assessed for at least one of the two measures (i.e. hippocampus and/or life events); a total of 54 subjects were assessed for both types of measures (i.e. hippocampus AND life events).
[b] Sample size of hippocampal volume only: Total group= 123; MZ=74 , DZ=49.
[c] Sample size of life events measures only: Total group= 57; MZ=30 , DZ=27.

BRAIN IMAGING

Magnetic resonance images were acquired on a Philips Intera 1.5 Tesla scanner (Philips, the Netherlands), with the following imaging parameters: T1-weighted 3D fast field echo scans with 160–180 contiguous coronal slices (echo time=4.6 ms, repetition time=30 ms, flip angle=30°, 1x1x1.2 mm^3 voxels) (van der Schot et al., 2009). MZ and DZ twins were randomly assigned to MRI slots, eliminating possible between-group biases due to scanner drifts.

Processing of brain images and hippocampal volumetric segmentation was performed with the FreeSurfer 5.1.0 structural imaging pipeline (http://surfer.nmr.mgh.harvard.edu/). Anatomic volume of the bilateral hippocampus was delineated using information on image intensity, probabilistic atlas location and spatial relationships between subcortical structures (Fischl et al., 2002; Fischl et al., 2004). All hippocampal segmentations were visually inspected to assure high quality data.

LIFE EVENTS AND DIFFICULTIES SCHEDULE (LEDS)

Life events were assessed with the investigator-based Bedford College Life Events and Difficulties Schedule (LEDS), a semi-structured interview assessing life events and long-term difficulties (Brown & Harris, 1978; Brown & Harris, 1989). The present study focused exclusively on life events. The LEDS collects detailed information about the event itself, the timing of its occurrence (date) and relevant contextual

information for each event. Based on the contextual information, the threat for each event is rated via standardized rating procedures. The threat score represents the severity of the event, ranging from mild (1) to severe (4). The contextual threat is conceptualized as: "What most people would be expected to feel about an event in a particular set of circumstances and biography, taking no account of what the respondent says either about his or her reaction or about any psychiatric or physical symptoms that followed it" (Brown & Harris, 1989). Severe events could be negative as well as positive, for example: moving to another country can be a very positive, but at the same time a stressful, life event. The interview covered life events from early childhood (after age 5) up to assessment. Life events were rated on a yearly basis. All interviewers and raters were trained by MH, who herself was trained by Brown and Harris who developed the LEDS. The events were rated from written transcripts by two independent raters who had not been involved in the interview. A panel consisting of five raters, including FB, SK and MH, reached consensus on the events that raised rating problems.

The cumulative life event load was calculated as the sum of the threat scores of all life events in year of MRI and all preceding years. We calculated three types of life event load. The total life event load; representing the sum of the threat scores of all life events (threat score 1 to 4), mild event load; representing the sum of the threat scores of all mild life events (threat scores 1 & 2) and severe event load; representing the sum of the threat scores of all severe life events (threat scores 3 & 4). Please refer to Supplementary Table S1 for some examples of life event load calculations.

STATISTICAL ANALYSES

Calculation of descriptive statistics and rendering of standardized residuals (obtained after regression on age, gender and intracranial volume (ICV) for hippocampal volume and on age only for life event measures) suitable for genetic model fitting were performed with the Statistical Package for the Social Sciences (IBM SPSS, release 21.0.0.0).

Genetic model fitting

To estimate relative genetic and environmental contributions to the association between hippocampal volume and life event measures, a bivariate continuous model was chosen and implemented in structural equation modeling software OpenMx (Kenny et al., 2009), running under the statistical programming environment R (R Development Core Team, 2008). A bivariate Cholesky decomposition was fitted to the standardized residuals of our hippocampal and life event measures (hippocampal volume was corrected for age, gender and ICV, and life event measures were corrected for age) to estimate additive genetic (A), and shared/common (C) and unique environmental (E) variance components of hippocampal volume and life event measures, and phenotypic, genetic and environmental overlap between these measures. The phenotypic correlation (r_{ph}), an index of association between phenotypes (e.g. hippocampal volume and severe life event load), was based on calculations of within-twin/between-trait correlations. Heritability (h^2) and influence of shared (c^2) and unique environment (e^2), as well as disentanglement of the observed correla-

tion between measures into genetic and environmental components, was based on polychoric cross-twin/within-trait and cross-twin/cross-trait correlations within MZ and DZ groups (Neale & Miller, 1997). The heritability of hippocampal volume and life event measures was determined within the bivariate model. A larger correlation between traits in MZ twins than in DZ twins suggests higher genetic contribution due to MZ twins being genetically identical, whereas DZ twins only share on average 50% of their segregating genes. If there is no difference between MZ and DZ correlations then a larger influence of shared environmental factors is more likely (Boomsma et al., 2002). The genetic (r_g) and shared (r_c) and unique environmental (r_e) correlations respectively indicate the degree of overlap in genes or shared and unique environment influencing phenotypes. The phenotypic correlation can be written as the sum of the genetic correlation weighted by the square root of the heritabilities of the two traits ($r_g * \sqrt{(h^2_{\text{hippocampus}})} * \sqrt{(h^2_{\text{life event measure}})}$) and the environmental correlations weighted by the square root of environmental variances associated with the two traits ($r_c * \sqrt{(c^2_{\text{hippocampus}})} * \sqrt{(c^2_{\text{life event measure}})}$; $r_e * \sqrt{(e^2_{\text{hippocampus}})} * \sqrt{(e^2_{\text{life event measure}})}$). These quantities are written as $r_{\text{ph-g}}$, $r_{\text{ph-c}}$ and $r_{\text{ph-e}}$ (Toulopoulou et al., 2007). In order to determine the model that best explained our data, we fitted different nested models and compared their goodness of fit using Akaike's Information Criterion (AIC). A saturated model in which means, variances and correlations are estimated freely served as a baseline model to which more restrictive models were compared. We performed an exhaustive analysis and determined the best fitting model (i.e. the model with the lowest AIC-value) of all the possible bivariate models (variable 1: hippocampal volume, variable 2: life event measure). This was done for each of the three bivariate relationships that we assessed (i.e. 'hippocampal volume and total life event load', 'hippocampal volume and mild life event load' and 'hippocampal volume and severe life event load'). Significance of parameter estimates and correlations within the best fitting model was determined based on 95% confidence intervals (CI) (Neale & Miller, 1997).

Correction for multiple comparisons

Correction for multiple testing was applied by dividing the alpha of 0.05 by the number of life event loads we assessed, which was 3. This resulted in a Bonferroni threshold for significance of $\alpha=0.05/3=0.017$. Significance at $\alpha=0.05$ and $\alpha=0.017$ is indicated in all tables and figures.

RESULTS

DEMOGRAPHIC INFORMATION AND DESCRIPTIVE STATISTICS

Table 1 shows the demographic information and mean values of hippocampal volume and life events measures for all groups. There were no differences in age, parental education, or own education between MZ and DZ twins. MZ twins had a significantly lower total life event load ($F [1, 55]=8.3$, $p=0.01$), mild life event load ($F [1, 55]=6.42$, $p=0.01$), and severe life event load ($F[1, 55]=4.67$, $p=0.04$) than DZ twins.

GENETIC MODEL FITTING

The best fitting models for the three bivariate comparisons are depicted in supplementary Fig. S1. Here, one model fitted best for the association of hippocampal volume with both total and severe life event loads. This model consisted of an A variance component for the hippocampus, a C variance component that was shared between the hippocampus and both total and severe life event loads, and two E variance components for the hippocampus and the life event load (total/severe) separately. For the association between hippocampal volume and mild life events, a model estimating A and E variance components for the hippocampus and C and E variance components for the mild life events but no shared factors between measures had the best fit. For the hippocampus, the mean variance components were calculated with the values obtained from the three bivariate models (i.e. 'hippocampus' vs 'total life event load', 'hippocampus' vs 'mild life event load', 'hippocampus' vs 'severe life event load').

Table 2 shows the MZ and DZ within-trait/cross-twin correlations, and variance components of genetic and environmental influences on all measures. Here, hippocampal volume showed high heritability (h^2: 57% to 81%) and low environmental influence in all three bivariate analyses whereas life event measures were not influenced by genes. The variance in total, mild and severe life event measures was attributable to shared and unique environmental factors (total, c^2: 52%, e^2: 48%; mild, c^2: 40%, e^2:60%; severe, c^2: 64%, e^2: 36%). Table 3 shows the MZ and DZ cross-trait/cross-twin correlations, as well as the phenotypic, genetic and environmental correlations between hippocampal volume and life events measures (see also Fig. 1). Hippocampal volume was significantly phenotypically associated with severe life event load (r_{ph}: -0.39), after Bonferroni correction for multiple comparisons. In the best-fitting model, this association was entirely explained by shared environmental factors. This indicates that shared events that influence twins similarly determined the association between smaller hippocampal volume and higher load of severe life events . In contrast, in the best fitting models, total and mild life event loads were not significantly phenotypically, genetically or environmentally associated with hippocampal volume.

Table 2. Within-trait/cross-twin correlations in MZ and DZ pairs measures (with 95% confidence intervals), and parameter estimates of genetic and environmental influences on all measures (with 95% confidence intervals).

	within-trait/cross-twin correlations			variance components		
	MZ	DZ		h^2	c^2	e^2
Measure[a]					%	
Hippocampal volume (in ml)[b, c]	**0.82**[†](0.67 to 0.91)	0.3 (-0.2 to 0.65)	(T)	**70**[†] (39 to 87)	11 (0 to 41)	**19**[†] (11 to 33)
			(M)	**81**[†] (68 to 89	-	**19**[†] (11 to 32)
			(S)	**57**[†] (27 to 81)	**24** (2 to 52)	**19**[†] (11 to 34)
Total life events, load[d]	0.34 (-0.43 to 0.79)	**0.63** (0.17 to 0.87)		0	**52**[†] (20 to 74)	**48**[†] (26 to 80)
Mild life events, load[d]	0.17 (-0.51 to 0.77)	0.37 (-0.17 to 0.75)		0	**40** (5 to 67)	**60**[†] (33 to 95)
Severe life events, load[d]	0.37 (-0.28 to 0.73)	**0.86**[†](0.64 to 0.95)		0	**64**[†] (37 to 81)	**36**[†] (19 to 63)

[†] Significant at α=0.017 (Bonferroni threshold), estimates in bold face are significant at α=0.05.
[a] Life event measures are corrected for age, and hippocampal volume is corrected for age, gender and intracranial volume.
[b] Sample size of hippocampal volume only: Total group= 123; MZ=74 , DZ=49.
[c] Heritability of the hippocampus was calculated for the 3 respective bivariate comparisons between hippocampal volume and life event loads. T: from total life events load, M: from mild life events load, S: from severe life events load.
[d] Sample size of life events measures only: Total group= 57; MZ=30 , DZ=27

Table 3. Cross-trait/cross-twin correlations in MZ and DZ twin pairs (with 95% confidence intervals), and phenotypic, genetic and environmental correlations with hippocampal volume (with 95% confidence intervals).

	cross-trait/cross-twin correlations		parameter estimates						
	MZ	**DZ**	r_{ph}	r_g	r_c	r_e	$r_{ph\text{-}g}$	$r_{ph\text{-}c}$	$r_{ph\text{-}e}$
Category (load) [a]	(n=29)	(n=25)							
Total life events	-0.31	-0.41	-0.24	-	-1	-	-	-0.24	-
	(-0.66 to 0.21)	(-0.7 to 0.04)	(-0.49 to 0.07)			(-1 to -1)			(-0.49 to 0.07)
Mild life events	-0.23	-0.26	-	-	-	-	-	-	-
	(-0.59 to 0.31)	(-0.59 to 0.19)							
Severe life events	-0.41	**-0.47**	**-0.39**[†]	-	**-**1	-	-	**-0.39**[†]	-
	(-0.7 to 0.02)	(-0.72 to -0.06)	(-0.61 to -0.11)			(-1 to -1)			(-0.61 to -0.11)

[†] Significant at α=0.017 (Bonferroni threshold), estimates in bold face are significant at α=0.05.
[a] Life event measures are corrected for age, and hippocampal volume is corrected for age, gender and intracranial volume.

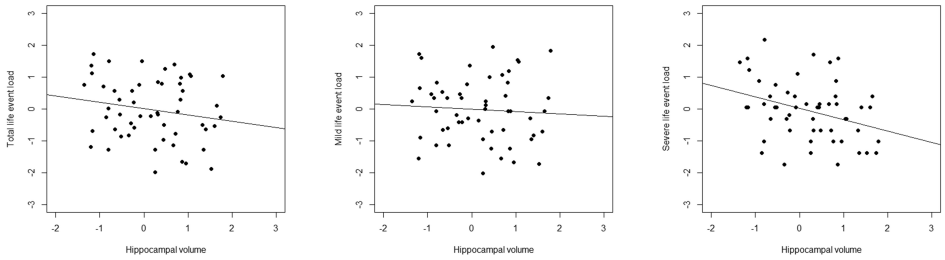

Fig. 1. Uncorrected hippocampal volumes and life event loads (total, mild and severe).

DISCUSSION

In this study, we assessed the influence of genes and environment on the relation between hippocampal volume and life events in healthy twins. Our main finding is that severe stressful life events are strongly associated with a smaller hippocampal volume and that this association is fully explained by shared environmental factors that influence twins similarly within the twin pairs. This suggests that severe shared events (e.g. divorce of parents, learning that a family member is seriously ill) may lead to a decrease in hippocampal volume. Despite study differences in MRI processing and assessment of life events, our findings are in line with earlier studies showing suggestive evidence for higher levels of stress to be associated with hippocampal volume loss (Gianaros et al., 2007; Papagni et al., 2011). We extend these findings by showing that stressful life events are associated with (smaller) hippocampal volume, especially when they are severe. In a previous twin study with combat exposed veterans who developed PTSD and their combat unexposed co-twins, Gilbertson et al. (2002) suggest that a smaller hippocampus may constitute a risk factor for the development of stress-related pathology. Particularly since both the combat exposed veterans with PTSD and their combat unexposed co-twins showed smaller hippocampi than combat exposed veterans without PTSD and their co-twins. However, in that study, the contribution of shared environmental factors that influence twins similarly on hippocampal volume and life stress was not assessed, so their involvement may have been overlooked.

The high heritability of hippocampal volume is in line with a previous study reporting a heritability of 69% (van Erp et al., 2004), although moderate genetic influence on the hippocampus has also been noted (Schmitt et al., 2007; Blokland, de Zubicaray, McMahon, & Wright, 2012). In contrast, life event measures showed large influences of shared and unique environmental factors with no influence of genetic factors. This contradicts previous studies that suggest that life events are influenced by genetic factors (see review by Kendler & Baker, 2007 and the study by Vinkenhuyzen, van der Sluis, de Geus, Boomsma & Posthuma, 2010). However, in those studies, the observed genetic influences were modest and mostly based on self-report measures of life events, which may have inflated heritability estimates (Vinkhuyzen et al., 2010). Furthermore, a strong influence of shared environmental factors on life events is to be expected as twins often experience the same life events and may be affected similarly by them. Nevertheless, significant unique environmental

influence on hippocampal and life event measures should be interpreted with caution as, in a twin model, this variance component also includes measurement error.
Our findings may be particularly relevant in psychiatry as hippocampal abnormalities have been shown in a number of stress-related psychiatric disorders, including bipolar disorder (Hajek, Kopecek, Hoschl & Alda, 2012), major depressive disorder (MDD) (Kempton et al., 2011), post-traumatic stress-disorder (PTSD) (Karl et al., 2006; Kuhn & Gallinat, 2013) and schizophrenia (Adriano, Caltagirone & Spalletta, 2012; Shepherd et al., 2012). Therefore, addressing the link between stress and hippocampal structure is important in understanding the mechanisms involved in serious mental illness.
Our findings show that it may be relevant to differentiate between life events with different levels of threat when investigating the relationship between stress and the brain. For future research, we also recommend to include functional measures of stress responsiveness, which allow for assessing potential mediatory effects of HPA axis reactivity (see Rabl et al., 2014)
There are several limitations that need to be taken into account when interpreting our findings. First, methodological limitations are a major issue when interpreting life events measures (Johnson, 2005). Regardless of the number of queries in an interview, people gradually forget life events (Paykel, 1997; Brown & Harris, 1982; Harris, 2001). The average participant in our sample had to report life events over a time span of 35 years. One could question the reliability of the LEDS when it is used retrospectively to collect lifetime life event data. However, the LEDS is probably more reliable compared to (retrospective) checklist inventories (Hillegers et al., 2004; Ormel, Oldehinkel & Brilman, 2001), as the LEDS minimizes recall bias by actively obtaining information in a very structured interview with detailed questions in ten domains. Second, using a relatively small sample size to estimate genetic and environmental sources of variance in bivariate designs reduces statistical power (Posthuma & Boomsma, 2000; Visscher, 2004). Furthermore, the confidence intervals that we reported were wide, diminishing precision of the extent of the estimates. The relatively small size of the sample may also have played a role in the finding that our MZ and DZ twins had a different number of life events. There is no a priori reason we can think of why this would be the case. However, we applied Bonferroni correction for multiple testing to ensure our estimates were robust. Third, females were overrepresented. Although a recent study showed that statistically controlling for ICV eliminates gender differences in hippocampal volume (among others) (Jancke, Merillat, Liem & Hanggi, 2015), other studies reported both larger (Inano et al., 2013) and smaller (Fjell et al., 2009) hippocampal volumes in females compared to males, after controlling for ICV. Therefore, in addition to including ICV as a covariate, we also controlled for gender to eliminate any influence it may have on hippocampal volume. Fourth, the twins have been selected previously as control subjects in studies with psychiatric patients, where stringent exclusion criteria with respect to the presence of axis I or II psychopathology were applied. Therefore, these subjects may not be representative of the general population. Fifth, it was not possible to ascertain the association between hippocampal volume and life events in different age groups in our sample, as doing so would have seriously affected statistical power. Nevertheless, it may be conceivable that shared environmental factors

have the largest influence during childhood and early adulthood, when the brain (and hippocampus) is also at its most plastic. Therefore, future studies could benefit from analyzing the influences of early versus late life events on hippocampal volume. Last, unfortunately our study does not allow for any statements regarding causality. Therefore, it remains unclear whether a smaller hippocampus is cause or consequence of severe stress. For example, a smaller hippocampal volume could also increase vulnerability to severe life events (but perhaps primarily those that are nonshared among twins), inflating the strength of the association (Gilbertson et al., 2002).

In conclusion, an association was found between smaller hippocampal volume and severe stressful life events, which was attributable to a complete overlap of shared environmental factors influencing both phenotypes. This indicates that severe events that are shared between twins predominantly contribute to both a smaller hippocampal volume and severe life events. In contrast, we did not observe a relationship between hippocampal volume and mild or total life event load. In this respect, our study highlights the importance of addressing the influence of shared environmental factors on the relation between stress and hippocampal volume, and to distinguish between mild and severe stressful life events in life event research.

SUPPLEMENTAL MATERIAL

Table S1. Example LEDS report and life event load calculation.

Description event	Threat score	Severity
Buying a house with partner	2	Mild
Daughter moves out of house	2	Mild
Borrowing 4000 euros	2	Mild
Winning 10.000 euros in lottery	3	Severe
House was broken into whilst on vacation, no expensive items missing	2	Mild
Broken arm, in cast for 6 weeks	3	Severe
Son & daughter in law announce pregnancy	1	Mild
Operation for hernia, unable to work for 3 months	4	Severe
Total life event load	19	
Mild life event load	9	
Severe life event load	10	

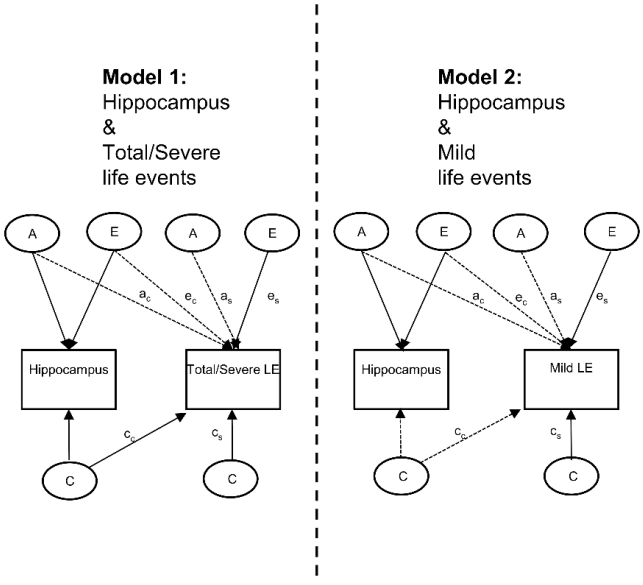

Fig. S1. Best fitting models for all three bivariate comparisons. Latent additive genetic (A), shared (C) and unique environmental (E) factors influence the hippocampus and life events, as indicated by arrows. Path coefficients (a_c and a_s) quantify the effects of genetic influences on life events, where a_c represents genetic influences that also influence the hippocampus and a_s represents genetic influences that are unique for life events. Similarly, path coefficients c_c, c_s, e_c and e_s quantify the effect of shared (C) and unique environmental (E) influences on phenotypes, respectively. Dotted arrows indicate genetic and environmental factors that would normally influence the hippocampus and life events under the full ACE-model but did not in the best fitting models at present.

CHAPTER 5

Is stress the common environmental factor explaining the relationship between pro-inflammatory monocytes and bipolar disorder? Results from the Dutch Bipolar Twin Study

Under review

S.M. Kemner, R.M. Brouwer, N.E.M. Haren, H.J. de Wit, R.S. Kahn, W.A. Nolen, H.A. Drexhage, M.H.J. Hillegers

ABSTRACT

We previously reported that the association of the monocyte pro-inflammatory state with bipolar disorder (BD) is primarily the result of a common shared environmental factor. One of these possible common environmental factors is stress. Both major and minor stressful life events can have direct adverse effects on a variety of immunological mechanisms have consequences for health

We collected detailed life event information across the life span using the LEDS. By using a trivariate twin model, including 49 bipolar twin pairs and 38 healthy twin pairs, we determined whether stressful life events explain the relationship between monocyte expression and bipolar disorder.

The results for trivariate modeling show that stressful life events cannot explain the common environmental association between BD and the pro-inflammatory gene expression of the monocytes. Interestingly life events were mainly influenced by genetic factors in bipolar disorder.

This study verifies our previous observation that common environmental factors determine monocyte activation in BD patients, yet shows that the experience of stressful life events is not one of these factors.

INTRODUCTION

Deviations of the immune system in psychiatric illness is increasingly acknowledged over the past 10 years. In a series of studies from our group we reported a higher expression of a set of 19 inflammation-related genes in circulating monocytes in a considerable proportion of patients with bipolar disorder (BD) (Drexhage et al., 2011). This same set of genes was found to be over-expressed in the majority of offspring of patients with BD, including those who had not yet developed a mood disorder but did so later in life (Padmos et al., 2008; Mesman et al., 2015).In a subsequent bipolar twin study we showed that the main portion of the total covariance between liability for BD and the pro-inflammatory state of monocytes could be attributed to common environmental factors and not to genetic effects or environmental factors unique to the individual (Padmos et al., 2009).

There are a number of environmental factors (e.g. infectious, dietary) that have been suggested to act as common environmental factors associated with both BD and pro-inflammatory monocyte activation (Padmos et al., 2009). One of these possible common environmental factors is the experience of stressful life events. In a review of animal and human studies on stress-associated immune dysregulation they concluded that both major and minor stressful life events can have direct adverse effects on a variety of immunological mechanisms and that these stressors have consequences for health (Padgett & Glaser, 2003). In addition, various studies have demonstrated that stressful life events play a role in the onset and course of BD (Bender & Alloy, 2011; Brown & Harris, 1989; Hillegers et al., 2004; Hlastala et al., 2000; Malkoff-Schwartz et al., 1998; Kemner et al., 2015).

In this study we explore in a post-hoc analysis, whether stressful life events, measured with the Life Events and Difficulties Schedule interview (Brown & Harris, 1989), explain the relationship between monocyte expression and BD using a trivariate twin model.

SUBJECTS AND METHODS

PARTICIPANTS

Participants were selected from the longitudinal twin study on BD of the University Medical Center Utrecht (UMCU), The Netherlands, and enrolled between 2001 and 2006. DNA fingerprinting using 9 to 11 high polymorphic microsatellite markers determined zygosity (Division of Genetics, University Medical Center Utrecht). The design of the study and the recruitment of the bipolar twin pairs with at least one twin with a DSM-IV bipolar I or II disorder as well as control twin pairs has been described in detail elsewhere (Van der Schot et al., 2009; Vonk, Van der Schot, Kahn, Nolen & Drexhage, 2007). All participants whose blood samples and/or a LEDS-interview were available, we included in the current analysis. Demographic information is displayed in Table 1.

In this study, 87 twin pairs were included: 49 bipolar twin pairs and 38 control twin pairs. Of the 49 bipolar twin pairs, 7 were monozygotic (MZ) concordant (both index twin and co-twin had bipolar disorder), 16 pairs were MZ discordant (co-twin did not have bipolar disorder), 4 pairs were dizygotic (DZ) concordant, and 22 pairs were

DZ discordant. All psychiatric diagnoses were confirmed with the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon & Williams, 1996) and the Structured Interview for DSM-IV Personality (Pfohl, Blum & Zimmerman, 1997). Current mood state was assessed using the Young Mania Rating Scale (YMRS; Young, Biggs, Ziegler & Meyer, 1978) and the Inventory for Depressive Symptomatology (IDS; Rush, Gullion, Basco, Jarret & Trivedi, 1996). At the time of the study, all patients were euthymic with an YMRS score of 4 or less and an IDS score of 12 or less. All patients were treated naturalistically.
The medical ethics review board of the UMCU approved the study and all participants gave written informed consent after full explanation of the study aims and procedures.

Table 1. Descriptives of Sample

	Bipolar Twin Pairs				Control Twin Pairs	
	MZ cc	MZ dc	DZ cc	DZ dc	MZ	DZ
Pairs (n)	7	16	4	22	19	19,5
LEDS interview & pro-inflammatory monocytes (n)	7	21	8	33	32	20
LEDS interview (n)	5	3	0	5	4	12
Pro-inflammatory monocytes (n)	2	8	0	6	2	7
Age, mean (range), y	34 (21-44)	38 (22-55)	43(33-50)	43(29-61)	39(21-57)	43(27-53)
Female sex, No (%)	8 (57)	22 (68)	6 (75)	28 (63)	32 (84)	24 (61)

Abbreviations: CC, concordant; DC, discordant; DZ, dizygotic; MZ, monozygotic

LIFE EVENT MEASURES

All subjects included in the current analysis were interviewed with the investigator-based Bedford College Life Events and Difficulties Schedule (LEDS; Brown & Harris, 1978, 1989). The LEDS is a semi-structured interview for assessing life events and long-term difficulties in adults. It collects detailed information about the event itself, the timing of its occurrence (date) and relevant contextual information for each event. Each event is categorized into one of ten domains, consisting of; education, work, reproduction, housing, money/possessions, crime/legal, health, martial/partner, other relationships, miscellaneous/death. Based on the contextual information, the threat for each event is rated via standardized rating procedures. The threat score represents the severity of the event, ranging from mild (1) to severe (4), hereby differentiating between mild life events (threat score 1 and 2) and more stressful life events (threat score 3 and 4). The contextual threat is conceptualized as: "What most people would be expected to feel about an event in a particular set of circumstances and biography, taking no account of what the respondent says either about his or her reaction or about any psychiatric or physical symptoms that followed it"[7]. Several studies have supported the reliability (e.g. interrater) and validity (e.g. multiple informant) of the LEDS in adults exhibiting a variety of psychiatric symptoms (Brown & Harris, 1978, 1989; Ormel, Oldehinkel & Brilman, 2001).

Only events occurring from the age of 5 years were included. All severe events (threat score 3 and 4) were defined by the extent they were related to the BD. To determine relatedness to the disorder each severe event was rated on a three point scale; 1) not related to BD; 2) possibly related to BD or; 3) clearly related to BD. Each life event was dated per year.
MH, who was trained by G.W. Brown and T.O. Harris who developed the LEDS, trained all interviewers and raters. The interviews were conducted at the participants home or at the UMCU. Two independent raters who had not been involved in the interviews rated the events. A panel consisting of the four raters (including SK and MH) reached consensus on the events that raised rating problems.

LABORATORY METHODS

Blood was collected in sodium-heparin tubes for immune cell preparation, isolation of monocytes and quantitative polymerase chain reaction (Q-PCR). From the heparinized blood, peripheral blood mononuclear cell suspensions were prepared using low-density gradient centrifugation. CD14-positive monocytes were isolated from frozen peripheral blood mononuclear cells by a magnetic cell sorting system (Miltenyi Biotec, Germany). The purity of monocytes was >95% (determined by morphological screening after Trypan Blue staining and fluorescent-activated cell sorting). RNA was isolated from monocytes as described earlier. To obtain complementary DNA (cDNA) for Q-PCR, 1 mg RNA was reversed-transcribed using the cDNA high-capacity cDNA Reverse Transcription kit (Applied Biosystems, USA). Q-PCR was performed with Taqman Universal PCR mastermix (Applied Biosystems, USA). All Taqman probes and consensus primers were pre-formulated and designed by Applied Biosystems (Assays on Demand). PCR conditions were 2 min at 50 xC, 10 min at 95 xC, followed by 40 cycles of 15 s at 95 xC, and finally 1 min at 60 xC. PCR amplification of the reference gene ABL was performed for each sample to allow normalization between the samples. ABL was chosen as a reference gene because it was previously shown that ABL was the most consistently expressed reference gene in haematopoietic cells (Beillard et al., 2003). The quantitative value obtained from Q-PCR is a cycle threshold (Ct). The fold change values between different groups were determined from normalized Ct values (Ct gene – Ct housekeeping gene), by the DDCt method.

STATISTICAL ANALYSIS

Life event load

The life event load represents the sum of the threat scores of all the life events occurring in each year.
We calculated three different life event load measures; (1) cumulative load (CL), i.e. the total life event load including all events (treat score 1 to 4) from age 5 till age at interview, (2) cumulative severe load (CSL) including only severe events (treat score 3 and 4) and (3) cumulative severe load including only severe events not related to the BD (CSL-exBP). All life event measures were treated as continuous variables. Life event loads are displayed in Table 2.

Table 2. Life event loads

	Bipolar Twin Pairs				Control Twin Pairs	
	MZ cc	MZ dc	DZ cc	DZ dc	MZ	DZ
Total life event load						
Mean (SD)	98.22 (37.67)[1]	96.07 (32.70)[1]	95.38 (19.44)[1]	98.56 (30.39)[1]	74.71 (25.26)	102.26 (21.84)
Min - Max	56 – 172	26 -184	77 - 125	59 - 164	34 - 137	55 - 149
Severe life event load						
Mean (SD)	33.56 (18.97)[1]	31.10 (11.8)[1]	36.13 (14.39)[1]	34.23 (17.90)[1]	12.32 (7.99)	22.04 (10.4)
Min - Max	13 – 76	13 - 57	12 - 52	15 - 89	0 - 33	3 - 43
Severe life event load without events related to bipolar disorder						
Mean (SD)	15 (9.12)	19.97 (11.64)	22.13 (11.18)	22 (11.98)		
Min - Max	3 – 34	3 - 50	9 - 43	3 - 56		

Abbreviations: CC, concordant; DC, discordant; DZ, dizygotic; MZ, monozygotic
Data not corrected for age
[1] significantly different at $p<.05$ from control twin pairs

Genes

All genes from the pro-inflammatory monocyte signature described in the previous study from our group (Padmos et al., 2009) were included separately into the model, using the fold change values (for the full list of genes see Supplementary table 1). The fold change values were determined from the normalized CT values (CT gene minus CT housekeeping gene) by the ΔΔCT method (2-ΔΔCT, User Bulletin 2, Applied Biosystems). To correct for interassay variance, we set the mean of the studied genes found in the healthy control index twins in the same assay for each gene to 1 (∑ΔCT healthy control index twins=; 2-0=1). The fold change values of the genes in patient monocytes were expressed relative to this set mean of 1 in the healthy controls.

Twin modelling

All statistical analyses were carried out using the OpenMx package (Kenny et al., 2009) in R statistical software (R Development Core Team, 2008). The life events variables were corrected for age at time of interview before they were entered in the genetic model. To estimate heritability, the twin model focuses on the difference in resemblance for a particular trait between monozygotic (MZ) twins who share (nearly) 100% of their genes to dizygotic (DZ) twin pairs who share on average 50% of their genes. Thus, if MZ twins resemble each other closer than DZ twins, there is reason to assume that the trait is heritable. A contribution of common environmental influences shared by members of a twin pair, is suggested when DZ correlations are larger than half the MZ correlations.

We used a two-phased approach. First, bivariate twin modelling was used to estimate the magnitude of the effects of A, C, and E on pro-inflammatory monocytes and their interdependence with BD. The same rationale as described above applied to

cross-trait cross-twin correlations can be used to establish whether an association between BD and monocytes is driven by genetic or environmental factors between the two traits. BD was modelled using a liability threshold model, assuming an underlying standard normal distribution for the liability to develop BD. Both affected and unaffected individuals have a liability to develop BD and once an individual crosses a threshold he/she is considered to have the disease. Since twin pairs in our sample were specifically selected for BD, we could not estimate heritability or prevalence in this sample. Hence, parameters were fixed to values that agree with the literature (h2=70%, c2=15%, e2=15%, prevalence=1%) (Smoller & Finn, 2003; Regeer et al., 2004; Ten Have, Vollebergh, Bijl & Nolen, 2002).

Pro-inflammatory monocytes were included per gene, as a continuous variable in the model. It has to be noted that the question of whether genetic or environmental sources drive the association between the liability to develop BD has been addressed before (Padmos et al., 2009), the difference being that in the previous analysis both variables were assumed to be ordinal, while the current analysis using a mixed ordinal-continuous variable approach that provides greater power.

In a second step, for those pro-inflammatory monocytes that shared a common environmental source with liability for BD, we investigated whether life events could be the shared common environment. To this end, we used a trivariate model in which the phenotypic, genetic and (common) environmental associations between liability for BD, pro-inflammatory monocyte and life events were included. We fitted this model for three types of life events variables: (1) CL, (2) CSL and (3) CSL-exBP. BD was included as a dichotomous variable, pro-inflammatory monocytes and life event measures, corrected for age, were included as continuous variables into the model.

RESULTS

PRO-INFLAMMATORY MONOCYTES AND BD

As expected based on the previous analyses, the results of analyses on pro-inflammatory monocytes and BD in the mixed ordinal-continuous model show that the correlation is mostly driven by common environmental factors, (Supplemental table 1). The expression of a subgroup of seven monocyte inflammatory genes (IL6, IL1B, PTGS2, TNF, CCL20, CXCL2, BCL2A1) had a significant common environmental correlation with BD after Bonferroni correction, see Table 3.

PRO-INFLAMMATORY MONOCYTES, BD & LIFE EVENTS

The results for trivariate modeling are displayed in Tables 3-5. The expression levels of the seven monocyte inflammatory genes were highly correlated (all r's > 0.8), and modelling results were very similar for the expression of these monocytes.

Total life events (severe & mild; cumulative load)

There were no associations between BD and cumulative life event load, including both mild and severe events, or the pro-inflammatory gene expression of the monocytes and all life events (Table 3).

Severe life events (cumulative load)

Severe life events correlated with both BD and the pro-inflammatory gene expression of the monocytes. Genes and unique environmental factors drove the association between BD and severe life events, indicating that severe life events cannot explain the common environmental association between BD and the pro-inflammatory gene expression of the monocytes (Table 4).
One gene of the subset of seven (CXCL2) suggested that the association between the pro-inflammatory gene expression of the monocytes and life events might be driven by common environmental factors (p=.04). However, this association was not related to BP (Table 4).

Severe life events not related to the disorder (cumulative load)

When life events related to the disorder were taken out of the analyses, the genetic association between life events and BD decreased to zero. Furthermore, the unique environmental correlation between liability for BD and life events became negative (Table 5).

Table 3. Modelling results for bipolar disorder, monocyte inflammatory genes and total life events (cumulative load)

	Correlation BP and life event load	Genetic correlation	Common environment correlation	Unique environment correlation	Correlation expression and life event load	Genetic correlation	Common environment correlation	Unique environment correlation
IL6	0.14 [-0.02 - 0.28]	0.40 [-1.00 - 1.00]	0.04 [-1.00 - 1.00]	-0.06 [-0.41 - 0.31]	0.00 [-0.19 - 0.20]	0.73 [-1.00 - 1.00]	0.00 [-1.00 - 1.00]	-0.06 [-0.31 - 0.19]
IL1B	0.14 [-0.01 - 0.29]	0.48 [-1.00 - 1.00]	0.02 [-1.00 - 1.00]	-0.05 [-0.41 - 0.31]	0.05 [-0.15 - 0.24]	0.46 [-1.00 - 1.00]	0.10 [-1.00 - 1.00]	-0.08 [-0.34 - 0.20]
PTGS2	0.14 [-0.02 - 0.29]	0.46 [-1.00 - 1.00]	-0.01 [-1.00 - 1.00]	-0.05 [-0.40 - 0.32]	0.12 [-0.94 - 0.32]	-0.45 [-1.00 - 1.00]	0.23 [-1.00 - 1.00]	0.04 [-0.23 - 0.31]
TNF	0.14 [-0.02 - 0.28]	0.39 [-1.00 - 1.00]	0.08 [-1.00 - 1.00]	-0.04 [-0.40 - 0.33]	-0.03 [-0.23 - 0.17]	-0.40 [-1.00 - 1.00]	-0.02 [-1.00 - 1.00]	-0.04 [-0.30 - 0.24]
CCL20	0.14 [-0.02 - 0.28]	0.44 [-1.00 - 1.00]	0.04 [-1.00 - 1.00]	-0.05 [-0.41 - 0.31]	0.02 [0.17 - 0.22]	0.41 [-1.00 - 1.00]	0.05 [-1.00 - 1.00]	-0.04 [-0.30 - 0.22]
CXCL2	0.14 [-0.01 - 0.29]	0.56 [-1.00 - 1.00]	-0.06 [-1.00 - 1.00]	-0.09 [-0.43 - 0.29]	0.10 [-0.01 - 0.29]	0.55 [-1.00 - 1.00]	0.18 [0.00 - 1.00]	-0.03 [-0.33 - 0.27]
BCL2A1	0.13 [-0.02 - 0.28]	0.40 [-1.00 - 1.00]	0.03 [-1.00 - 1.00]	-0.06 [-0.41 - 0.30]	0.02 [-0.18 - 0.22]	0.85 [-1.00 - 1.00]	-0.03 [-1.00 - 1.00]	-0.12 [-0.40 - 0.19]

Heritability of all life events from this model: $h^2 = 0.16$ [0 - 0.71]; $c^2 = 0.37$ [0 - 0.65]; $e^2 = 0.46$ [0.26 - 0.72]

Table 4. Modelling results for bipolar disorder, monocyte inflammatory genes and severe life events (cumulative load)

	Correlation BP and life event load	Genetic correlation	Common environment correlation	Unique environment correlation	Correlation expression and life event load	Genetic correlation	Common environment correlation	Unique environment correlation
IL6	0.42[2] [0.27 - 0.54]	0.31[1] [0.04- 0.59]	0.99 [-1.00 - 1.00]	0.50[1] [0.10 - 0.79]	0.21[1] [0.01 - 0.38]	0.96 [-1.00 - 1.00]	0.59 [-1.00 - 1.00]	-0.12 [-0.38 - 0.17]
IL1B	0.42[2] [0.28 - 0.55]	0.32[1] [0.05- 0.60]	0.99 [-1.00 - 1.00]	0.49[1] [0.09 - 0.78]	0.21[1] [0.02 - 0.39]	0.98 [-1.00 - 1.00]	0.70 [-1.00 - 1.00]	-0.05 [-0.35 - 0.27]
PTGS2	0.42[2] [0.28 - 0.54]	0.32[1] [0.06- 0.59]	0.99 [-1.00 - 1.00]	0.47[1] [0.07 - 0.77]	0.27[1] [0.06 - 0.45]	0.38 [-1.00 - 1.00]	0.89 [-1.00 - 1.00]	-0.05 [-0.35 - 0.27]
TNF	0.42[2] [0.28 - 0.54]	0.31[1] [0.05- 0.59]	0.99 [-1.00 - 1.00]	0.50[1] [0.09 - 0.79]	0.17 [-0.03 - 0.36]	-0.09 [-1.00 - 1.00]	0.73 [-1.00 - 1.00]	-0.08 [-0.37 - 0.23]
CCL20	0.42[2] [0.28 - 0.55]	0.32[1] [0.06- 0.59]	0.99 [-1.00 - 1.00]	0.48[1] [0.08 - 0.77]	0.20[1] [0.01 - 0.38]	1.00 [-1.00 - 1.00]	0.65 [-1.00 - 1.00]	-0.13 [-0.41 - 0.18]
CXCL2	0.43[2] [0.29 - 0.55]	0.33[1] [0.06- 0.61]	0.99 [-0.44 - 1.00]	0.46[1] [0.06 - 0.77]	0.24[1] [0.05 - 0.41]	0.33 [-1.00 - 1.00]	0.85[1] [0.00 - 1.00]	-0.04 [-0.38 - 0.31]
BCL2A1	0.41[2] [0.27 - 0.54]	0.33[1] [0.07- 0.60]	0.95 [-1.00 - 1.00]	0.48 [1] [0.07 - 0.78]	0.19 [-0.01 - 0.37]	0.71 [-1.00 - 1.00]	0.51 [-1.00 - 1.00]	-0.09 [-0.41 - 0.27]

Heritability of severe life events from this model: $h^2 = 0.73$ [0.39 - 0.87]; $c^2 = 0.08$ [0 - 0.39]; $e^2 = 0.18$ [0.10 - 0.33].

[1] significant at 0.05 level

[2] significant after Bonferroni correction ($p < 0.05/22$)

Table 5. Modelling results for bipolar disorder, monocyte inflammatory genes and severe life events without events related to bipolar disorder (cumulative load)

	Correlation BP and life event load	Genetic Correlation	Common environment correlation	Unique environment correlation	Correlation expression and life event load	Genetic correlation	Common environment correlation	Unique environment correlation
IL6	0.03 [-0.13 - 0.18]	-0.07 [-1.00 - 0.29]	0.93 [-1.00 - 1.00]	-0.42[1] [-0.70 - -0.05]	0.05 [-0.16 - 0.24]	-0.30 [-1.00 - 1.00]	0.59 [-1.00 - 1.00]	0.04 [-0.22 - 0.31]
IL1B	0.03 [-0.13 - 0.18]	-0.06 [-1.00 - 0.29]	0.94 [-1.00 - 1.00]	-0.41[1] [-0.70 - -0.03]	0.06 [-0.14 - 0.26]	-0.85 [-1.00 - 1.00]	0.25 [-1.00 - 1.00]	0.03 [-0.26 - 0.32]
PTGS2	0.03 [-0.13 - 0.18]	-0.06 [-1.00 - 0.30]	0.95 [-1.00 - 1.00]	-0.42[1] [-0.70 - -0.05]	0.12 [-0.98 - 0.33]	-0.77 [-1.00 - 1.00]	0.46 [-1.00 - 1.00]	0.04 [-0.25 - 0.33]
TNF	0.03 [-0.13 - 0.18]	-0.05 [-1.00 - 0.30]	0.95 [-0.19 - 1.00]	-0.44[1] [-0.71 - -0.07]	0.02 [-0.18 - 0.23]	-0.88 [-1.00 - 1.00]	0.44 [-0.24 - 1.00]	-0.07 [-0.34 - 0.21]
CCL20	0.03 [-0.13 - 0.18]	-0.05 [-1.00 - 0.31]	0.88 [-1.00 - 1.00]	-0.42[1] [-0.71 - -0.05]	0.04 [-0.16 - 0.24]	-0.45 [-1.00 - 1.00]	0.15 [-1.00 - 1.00]	-0.05 [-0.32 - 0.23]
CXCL2	0.03 [-0.12 - 0.19]	-0.05 [-0.52 - 0.29]	0.98 [-0.11 - 1.00]	-0.41[1] [-0.70 - -0.04]	0.06 [-0.14 - 0.25]	-1.00 [-1.00 - 1.00]	0.54 [-0.13 - 1.00]	0.09 [-0.24 - 0.38]
BCL2A1	0.03 [-0.13 - 0.18]	-0.05 [-0.55 - 0.28]	0.94 [-1.00 - 1.00]	-0.401 [-0.70 - -0.03]	0.00 [-0.20 - 0.20]	-1.00 [-1.00 - 1.00]	0.42 [-1.00 - 1.00]	0.12 [-0.21 - 0.41]

Heritability of serious life events without events related to bipolar disorder from this model: $h^2 = 0.52$ [0.02 - 0.79]; $c^2 = 0.17$ [0 - 0.52]; $e^2 = 0.31$ [0.17 - 0.59].
[1] significant at 0.05 level

DISCUSSION

In this study we explored in a post-hoc analysis whether stressful life events as common environmental factor explains the relationship between monocyte inflammatory gene expression and BD using a trivariate twin model.

First, we replicated the results of the original analyses by Padmos et al. (2009) using a different model, showing that the correlation between the pro-inflammatory gene expression in monocytes and BD is strongly influenced by common environmental factors.

Next, we ran the model per gene and detected a subgroup of seven genes which had a significant common environmental correlation with BD. These seven genes belong to a cluster of around 13 mutually correlating inflammatory genes which we have consistently identified as over-expressed in a number of studies on activated monocytes of patients with various auto-inflammatory conditions, including BD (Drexhage et al., 2011; Mesman et al., 2015; Bergink et al., 2013) and major depression (Carvalho et al., 2014; Grosse et al., 2015). This gene cluster (called sub-cluster 1) codes for inflammatory compounds or compounds playing a role in the production of inflammatory cytokines (ATF3, BCL2A1, CCL20, CXCL2, DUSP2, EREG, IL-1B, IL-6, PDE4B, PTGS2, PTX3, TNF, TNFAIP3). In addition to this cluster of genes there is another cluster of 7 genes often over-expressed in activated monocytes of patients with auto-inflammatory conditions; this cluster of genes codes for compounds playing a role in the adhesion, shape change and cell differentiation of the monocytes and is called sub-cluster 2 (CCL2, CCL7, CDC42, DHRS3, EMP1, MAPK6, NAB2, PTPN7, STX1A). It is worth noting that the genes of this latter cluster did not have a significant common environmental correlation with BD.

We found genetic correlations between BD and life events, but these associations disappeared when life events related to the disorder were disregarded. The disappearance of the genetic association suggests that life events related to the BD also occur for the co-twin (more so for MZ than DZ twins). This is a possible effect of the co-twin often being involved in the severe events of the affected twin (e.g. co-twin being involved in admission in a psychiatric hospital during a severe mood episode). Interestingly, we did not find evidence for our assumption that stressful life events would at least partially explain the association between monocyte inflammatory gene expression and BD.

In fact, we found that life events themselves were mainly influenced by genetic factors in this cohort enriched for BD. This is in line with Kendler & Baker (2007) who reviewed 55 studies that measured the genetic influence on a wide variety of environmental measures, such as general and specific life events, parenting as reported by child or parent, family environment, social support, peer interactions, and marital quality. They found that these environmental measures are indeed influenced by genetic factors. A more recent study replicated this (Vinkenhuyzen, van der Sluis, de Geus, Boomsma & Posthuma, 2010), but stated that the influences of genetic factors were small and that the reviewed findings were often inconsistent. All above mentioned studies concerned healthy twin pairs and indeed the heritability of life events was low when we investigated the healthy co-twins only (Bootsman et al., 2016). Adding bipolar patients would probably add more variation to life events measures,

which increases the probability of detecting a genetic component; especially since we found BD and serious life events to be genetically related. Our finding that the influence of genetic factors diminished when disorder related events were taken out of the analysis, supports this idea. The unique environmental correlation between liability for BD and the serious life events not related to the disorder were negative in this model. This implies that subjects at risk for BD experience less life events not related to the disease than healthy subjects. Possibly bipolar patients seek out environments in which they are less exposed to stressful events, but it should be emphasized that this speculation at this point.

Interestingly, a recent study by Becking et al. (2015) investigated whether increased inflammatory gene expression was a trait or a state marker in patients with BD. They reported that inflammatory gene expression is elevated in BD patients with an episode compared to healthy controls as well as compared to euthymic BD patients. Longitudinal analyses on this small subsample showed that within patients the presence of a mood episode determined whether inflammatory gene expression was elevated. Since all our patients were euthymic at the time of the study the influence of mood state on our results remains unclear.

If life events are not the common environmental factor we are looking for, what could be the alternative possibilities? Padmos et al. (2009) had already put forward several explanations other than stress to explain the common environmental factor explaining the correlation between monocytes over expressing genes involved in the production of pro-inflammatory cytokines and BD. An environmental factor that has gained interest over the last few years is an inflammatory influence exerted during fetal life due to stress or infection of the mother during pregnancy. Infection but also environmental stressors during gestation/early life are capable to activate microglia, perturbing neuronal development, thereby setting the stage for vulnerability for later psychiatric disorders. A second hit, such as endocrine changes, stress, or infection, could further activate the microglia, leading to functional abnormalities of the neuronal circuitry in the brain and mood episodes (Bergink et al., 2013). However early dietary influences or gut microbioma shared between the members of a twin pair should also be considered as environmental factor linking BD and inflammatory monocytes, since both are capable of inducing altered inflammatory set points of the monocyte/macrophage system. Further studies are needed to determine the common eliciting immune factor(s) and to provide a possible marker for treatment of both BD and immune abnormalities.

LIMITATIONS

There are several limitations that need to be taken into account when interpreting our findings.

First, all our patients were naturalistically treated, which resulted in a variety of medication types that are known to influence inflammatory gene expression. Especially lithium and antipsychotics have been reported to have a dampening effect on gene expression (Padmos et al., 2008; Drexhage et al., 2010).

Second, although most analysis yielded significant results, we have a small sample size consisting of patients with bipolar I as well as bipolar II disorders. The small sample size did not allow us to compare the two subtypes.

Third, the average participant in our sample had to report life events over a time

span of 35 years. One could question the reliability of the LEDS when it is used retrospectively to collect lifetime life event data. Most studies restrict the reporting of life events to a 12-month period. However, the LEDS is probably more reliable compared to (retrospective) checklist inventories (Hillegers et al., 2004; Ormel et al., 2001), as the LEDS minimizes recall bias; information is actively obtained in a very structured interview by detailed questions in ten domains. Furthermore, there is evidence that recall bias is more pronounced for minor events, suggesting that major life changes are under less influence of recall bias (Funch & Marshall, 1984), for this reason we included only the severe events in the main analysis.

CONCLUSION

This study verifies our previous observation that common environmental factors determine monocyte activation in BD patients, yet shows that the experience of stressful life events is not one of these factors. Interestingly life events were mainly influenced by genetic factors in bipolar disorder.

Supplemental Table 1. Modelling results for monocyte inflammatory genes and bipolar disorder

	Correlation BP and expression	Genetic correlation	Common environmental correlation	Unique environmental correlation	h2	c2	e2
PDE4B	**0.19** [0.04 - 0.34]	1.00 [-1.00 - 1.00]	0.39 [-0.17 - 0.98]	0.12 [-0.31 - 0.50]	0.00 [0.00 - 0.15]	**0.87*** [0.73 - 0.92]	0.12 [0.08 - 0.19]
IL6	**0.27*** [0.12 - 0.41]	1.00 [-1.00 - 1.00]	**0.56** [0.10 - 1.00]	0.22 [-0.13 - 0.57]	0.00 [0.00 - 0.05]	**0.91*** [0.85 - 0.94]	0.09 [0.06 - 0.13]
IL1B	0.30* [0.15 - 0.44]	1.00 [-1.00 - 1.00]	**0.65** [0.13 - 1.00]	0.30 [-0.10 - 0.64]	0.00 [0.00 - 0.09]	**0.89*** [0.80 - 0.93]	0.10 [0.07 - 0.16]
PTX3	**0.24*** [0.09 - 0.38]	1.00 [-1.00 - 1.00]	0.47 [-0.04 - 0.98]	0.04 [-0.33 - 0.39]	0.01 [0.00 - 0.09]	**0.87*** [0.78 - 0.91]	0.13 [0.08 - 0.19]
PTGS2	**0.25*** [0.09 - 0.38]	-1.00 [-1.00 - 1.00]	**0.86** [0.15 - 1.00]	0.21 [-0.18 - 0.55]	0.01 [0.00 - 0.23]	**0.74*** [0.51 - 0.83]	0.25 [0.17 - 0.37]
TNF	**0.23** [0.08 - 0.37]	-1.00 [-1.00 - 1.00]	**0.72** [0.12 - 1.00]	0.25 [-0.14 - 0.60]	0.01 [0.00 - 0.14]	**0.80*** [0.65 - 0.87]	0.19 [0.13 - 0.29]
TNFAIP3	**0.26*** [0.11 - 0.40]	0.26 [-1.00 - 1.00]	0.46 [-0.22 - 1.00]	0.31 [-0.09 - 0.65]	0.06 [0.00 - 0.35]	**0.76*** [0.49 - 0.87]	0.18 [0.11 - 0.28]
CCL2	**0.24*** [0.09 - 0.38]	0.67 [-1.00 - 1.00]	0.31 [-0.52 - 1.00]	0.08 [-0.31 - 0.44]	0.05 [0.00 - 0.46]	**0.66*** [0.29 - 0.79]	0.29 [0.17 - 0.43]
CCL7	**0.24*** [0.09 - 0.38]	0.24 [-1.00 - 1.00]	0.54 [-0.21 - 1.00]	-0.09 [-0.48 - 0.32]	0.17 [0.00 - 0.49]	**0.67*** [0.37 - 0.85]	0.16 [0.09 - 0.27]
CCL20	**0.30*** [0.15 - 0.44]	1.00 [-1.00 - 1.00]	**0.67** [0.18 - 1.00]	0.22 [-0.18 - 0.58]	0.00 [0.00 - 0.07]	**0.91*** [0.84 - 0.94]	0.09 [0.06 - 0.14]
CXCL2	**0.28*** [0.13 - 0.42]	1.00 [-1.00 - 1.00]	**0.73** [0.12 - 1.00]	0.05 [-0.35 - 0.44]	0.00 [0.00 - 0.21]	**0.84*** [0.64 - 0.90]	0.16 [0.10 - 0.24]
CCR2	-0.15 [-0.31 - 0.03]	-1.00 [-1.00 - 1.00]	-0.32 [-1.00 - 0.83]	0.45 [-0.07 - 0.94]	0.04 [0.00 - 0.46]	**0.51** [0.14 - 0.69]	0.44 [0.28 - 0.66]
CDC42	0.14 [-0.01 - 0.29]	0.14 [-1.00 - 1.00]	0.26 [-1.00 - 1.00]	0.08 [-0.30 - 0.45]	0.22 [0.00 - 0.73]	0.47 [0.00 - 0.74]	0.31 [0.19 - 0.50]

BCL2A1	**0.26*** [0.11 - 0.39]	-0.49 [-1.00 - 1.00]	**0.77** [0.08 - 1.00]	0.17 [-0.23 - 0.53]	0.01 [0.00 - 0.33]	**0.77*** [0.47 - 0.85]	0.23 [0.13 - 0.34]
EMP1	0.09 [-0.06 - 0.24]	1.00 [-1.00 - 1.00]	0.12 -0.80 - 1.00]	0.16 [-0.27 - 0.55]	0.00 [0.00 - 0.29]	**0.60** [0.31 - 0.73]	0.40 [0.27 - 0.56]
DUSP2	**0.21** [0.06 - 0.36]	-0.07 [-1.00 - 1.00]	0.90 [-0.41 - 1.00]	0.15 [-0.27 - 0.51]	0.32 [0.00 - 0.75]	0.38 [0.00 - 0.73]	0.30 [0.19 - 0.47]
ATF3	**0.23** [0.08 - 0.37]	-1.00 [-1.00 - 1.00]	0.84 [-0.12 - 1.00]	0.32 [-0.08 - 0.65]	0.01 [0.00 - 0.50]	**0.59** [0.14 - 0.72]	0.40 [0.26 - 0.56]
NAB2	0.01 [-0.15 - 0.16]	1.00 [-1.00 - 1.00]	-0.41 [-1.00 - 0.42]	0.24 [-0.15 - 0.59]	0.01 [0.00 - 0.35]	**0.63*** [0.31 - 0.75]	0.36 [0.24 - 0.51]
MAPK6	0.15 [-0.00 - 0.29]	0.03 [-1.00 - 1.00]	0.53 [-0.47 - 1.00]	-0.03 [-0.40 - 0.34]	0.22 [0.00 - 0.70]	**0.49** [0.05 - 0.75]	0.29 [0.18 - 0.48]
FCAR	**0.26*** [0.11 - 0.40]	-1.00 [-1.00 - 1.00]	0.80 [-0.02 - 1.00]	0.15 [-0.27 - 0.51]	0.00 [0.00 - 0.32]	**0.65*** [0.34 - 0.76]	0.35 [0.24 - 0.50]
PTPN7	0.14 [-0.01 - 0.28]	0.59 [-1.00 - 1.00]	-0.18 [-1.00 - 1.00]	-0.16 [-0.50 - 0.22]	0.20 [0.00 - 0.73]	0.46 [0.00 - 0.70]	0.34 [0.21 - 0.53]

Bold = significant at 0.05 level

* significant after Bonferroni correction ($p < 0.05/22$)

CHAPTER 6

Summary and Discussion

INTRODUCTION

The general aim of this study was to expand our knowledge on the role of life events as potential risk factor in the onset and course of bipolar disorder (BD), a complex and multifactorial disease with genetic and environmental factors contributing to its clinical expression.

MAIN FINDINGS

CHAPTER 2

In **chapter 2** we aimed to clarify the role of life events on first and recurrent admissions in bipolar patients, including the role of the kindling hypothesis in this relation. Our study showed that life event load, operationalized as the number and severity of life events, had an impact on both first and recurrent admissions in bipolar patients. This has also been found in previous studies (Bender & Alloy, 2011; Hunt, Bruce-Jones, & Silverstone, 1992; Kessing, Andersen, & Mortensen, 1998; Kessing, Agerbo, & Mortensen, 2004). Remarkably this effect was not influenced by events related to the illness which suggests that the effect of life events is independent of the disorder itself.

In addition, we demonstrated that the risk for admission increases with each subsequent admission. In other words, the risk of readmission increases as a function of the number of previous admissions. Given our finding that the risk of getting admitted is independent of events related to the disorder, such as admissions, the association between the number of previous admissions and the increased risk of readmission might be interpreted as an indicator for illness severity. Moreover, this finding also suggests a possible kindling effect (e.g. life events play a greater role in the onset of initial episodes than in subsequent later episodes, which can even occur more or less spontaneously; Post, 1992, Post, 2016). However, this finding did not reach statistical significance to support this indication of kindling.

A decay model was applied to the life event data, implying that the presumed effect of life events diminishes over time. The decay model of 25% best explained the impact of stressful life events on the onset of mood disorders, meaning that although the influence of stressful life events accumulates over time, this effect also diminishes with 25% per year. In other words, there is a limited expiration of the effect of stressful life events over time. The underlying mechanisms that cause this decay are unknown; a possible explanation lies in the interaction of life stress with coping strategies and temperament. Coping strategies, depending if they are constructive or maladaptive, can increase or decrease levels of stress and therefore influence the association between stress and the onset of mood episodes. In addition, temperamental traits (innate aspects of personality) influence individual coping styles and in that way modify the impact of stressful life events on mood episode onset (Compas, Connor-Smith, & Jaser, 2004). In the following chapter, we have studied the influence of these psychosocial factors in more detail.

Overall this study demonstrated a significant impact of stressful life events on both first and recurrent admissions in bipolar patients and this effect appeared to be independent of events related to the illness.

CHAPTER 3

In **chapter 3** we studied the effect of stressful life events on the onset of a first and recurrent mood episodes in children of parents with BD (bipolar offspring), as well as the impact of temperament, coping and parenting styles on this association. Results of this study indicate that severe life events were associated with an increased risk for first and, although less pronounced, subsequent mood episodes. The study replicated findings of the second assessment at 14 months follow-up of the Dutch Bipolar Offspring Study (Hillegers et al., 2004). Here, we also applied several decay models and similar to chapter 2 the history of life events did not constitute a pure accumulative load as a natural decay effect of 50-75% per year was observed.
We also found a large confounding effect for the number of previous mood episodes, suggesting a possible kindling effect for mood disorders among bipolar offspring. Passive coping style increased the risk of first mood episode onset and recurrent episodes, but also had a confounding effect on the association between life events and mood episode onset, suggesting that possibly the way offspring handle stress may be an important target for intervention. Harm avoidance temperament was found to be associated with mood episode recurrence and may possible indicate a general risk factor for mood recurrence.
Overall, this study found several risk factors to be associated with mood episode onset and recurrence in different ways and provides targets for early intervention.

CHAPTER 4

In **chapter 4**, we describe a study assessing the association between hippocampal volume and life events in a sample of healthy twins. The hippocampus is involved in negative feedback signalling to the hypothalamus during the hypothalamic-pituitary-adrenal (HPA) activated response to environmental stress, a process which results in secretion of glucocorticoids that are vital to short-term survival (Jankord & Herman, 2008). Chronic stress could disrupt the feedback signalling and consequent glucocorticoid toxicity in the hippocampus itself which, as a result may show atrophy and diminished neurogenesis (Sapolsky et al, 1990; Brown et al, 2004; Conrad, 2006; Mirescu & Gould, 2006; Gianaros et al; 2007; McEwen, 2007). Therefore, the association between hippocampal volume and life events was assessed. Moreover, the extent to which genes and environment influenced the association was determined.
The results showed that smaller hippocampal volume was related to higher severe life event load, which is in line with previous studies indicating an association between hippocampal volume loss and higher levels of stress (Gianaros et al, 2007; Papagni et al 2011). Moreover, these findings were extended in the present study as we showed that a severe life event load was associated with smaller hippocampal volume and not total or mild life event loads. Furthermore, environmental factors that were shared between twins fully explained the association between smaller hippocampal volume and a high level of life event load. This suggests that severe life events experienced by both twins, for example the loss of a parent, may have the largest impact on the hippocampus. In addition, hippocampal volume was primarily influenced by genes whereas life event measures were predominantly influenced by shared and unique environmental factors.

Based on these results, it is recommended to assess the influence of life events that affect twins similarly and to distinguish between severe and mild life events when assessing the relation between hippocampal volume and life stress.
Addressing the link between stress and hippocampal structure is valuable in expanding our understanding of the mechanisms involved in severe mental illness. Hippocampal abnormalities have been shown in a number of stress-related psychiatric disorders, including BD (Hajek et al, 2012). Unfortunately, we were unable to expand this study with the inclusion of bipolar twins, due to the small sample size.
Overall, this study in healthy twins demonstrated that smaller hippocampal volume was strongly associated with a high load of stressful life events, shared between twins. The heritability of hippocampal volume was high, whereas the life event load was predominantly influenced by shared and unique environmental factors.

CHAPTER 5

The study described in **chapter 5** explored the role of life events in the association between pro-inflammatory monocytes and BD in twins. We found genetic correlations between BD and life events, but these associations disappeared when life events related to the disorder were disregarded. The disappearance of the genetic association suggests that life events related to the BD also occur for the co-twin (more so for MZ than DZ twins). This is a possible effect of the co-twin often being involved in the severe events of the affected twin (e.g. co-twin being involved in admission in a psychiatric hospital during a severe mood episode).
Interestingly, we did not find evidence for our assumption that stressful life events would at least partially explain the association between monocyte inflammatory gene expression and BD. New findings indicate that affective episodes may be classified as pro-inflammatory states where different levels of concentrations of monocytes were found between the different mood episodes, suggesting that mania and depression are associated with a proinflammatory state (Becking et al., 2015).
Overall, this study verifies our previous observation that common environmental factors determine monocyte activation in BD patients, yet showing that the experience of stressful life events is not one of these factors. Interestingly, life events were mainly influenced by genetic factors in BD.

METHODOLOGICAL CONSIDERATIONS

The findings of the studies described in this thesis should be viewed in light of a few limitations. For all chapters the relatively small sample size and the life event methodology are the most prominent factors to be considered when interpreting our results. Within each chapter the strengths and limitations of the specific chapters are described in detail.
The results described in this thesis should thus only carefully be generalized to the total population of bipolar patients because both designs might be prone to specific selection biases described below.

Offspring Design

The study of children from a parent with BD using a longitudinal study design is an elegant and valid method to study the familial transmission of BD and the early trajectories of BD. Bipolar offspring represent a unique population at increased risk as they do not only inherit a genetic risk, but might also experience impaired family functioning and parenting skills as a result of parental mood episodes. In addition, assortative mating is common in these families resulting in an increased level of familial loading for (bipolar and unipolar depressive) mood disorders and other mental disorders as well as environmental complexities (Goodwin & Jamison, 2007).
In case of the Dutch Bipolar Offspring Study the cohort may not be fully generalizable to bipolar offspring in general. The majority (73%) of the families included in our cohort were recruited via the Dutch Patient Association for Manic Depressives and Relatives (Nederlandse Vereniging voor Manisch-Depressieven en Betrokkenen; VMDB). These families (both patients and their families) may be a selection of better functioning, better informed, motivated and a treatment seeking patient population. However, within our own sample, the families recruited via the VMDB did not differ in illness characteristics (number of hospitalizations, number of manic episodes and age of onset), socio-economic status and divorce rate, from the families recruited via outpatient clinics (Mesman, 2015; Hillegers, 2007).

Twin Design

Our twin sample is not a population-based twin sample, but a selected subgroup of bipolar twins and healthy (control) twins in the Netherlands. For instance, more female bipolar twins (68%) and female control twins (71%) than male twins participated in our study. This is probably a result of selection, as in the general population the prevalence of BD is the same in men and women (Weissman et al, 1996) On the other hand, the whole sample of affected twins can be considered representative as the concordance rates in our sample of 53 affected twins, 55% for MZ twins and 24% for DZ twins, is comparable with other reported concordance rates for BD (McGuffin et al). Also the healthy control twins form a selection, as they were selected for lifetime absence of any psychiatric disorder as well as for absence of a family history of any major psychiatric disorder.

COMPLEXITY OF RISK FACTORS IN THE STUDY TO BIPOLAR DISORDER

BD is a complex multifactorial illness. Empirical and theoretical work on BD has been going back and forth between psychological and biological conceptualizations. With the rise of family- and twin studies the interplay between environmental and genetic factors became a point of focus and has given rise to new and valuable insights into the mechanisms that contribute to psychiatric illness development. While our research contributes to a better understanding of gene-environment interaction (specifically life events) in BD, we should be aware that this is simplified representation of reality.
We know that the study of environmental risk factors poses various challenges. Many different environmental factors have been suggested to influence onset of

psychiatric disorders, e.g. urbanity, diet, but also traumatic events or pre-natal and perinatal conditions somatic etc. (Rutter et al. 2001; Brown & Harris, 1978; Bergink et al., 2013).
An additional complicating factor is that in most cases environmental variables are not purely environmental, but act under the influence of both genetic and environmental factors. In our studies, we aimed to measure stress by using life events as an environmental factor, but simultaneously showed that they are (at least moderately) influenced by genetic factors (**chapter 4 – 5**). This is not a recent finding as it was shown by Kendler and Baker (2007) in a review of 55 studies that measured the genetic influence on a wide variety of environmental influences, such as general and specific life events, parenting as reported by child or parent, family environment, social support, peer interactions, and marital quality. They showed that these environmental measures are indeed at least partly, varying from small to moderate, influenced by genetic factors. A more recent study replicated this finding (Vinkenhuyzen et al., 2010) , but explicitly stated that these influences were small and the reviewed findings were often inconsistent. Therefore, heritability of environmental factors could introduce a bias to models that treat these factors as purely environmental in origin and may therefore impede our understanding of individual differences in complex traits.
In addition to showing that life events are influenced by genetic factors, psychosocial factors also influence the effect of life events on the onset and course of BD (**chapter 3**). Passive coping style was involved in the effect of life events, but also contributed to the onset and recurrence of bipolar episodes. This is a good reflection of the complexity in the study to risk factors. Even though a direct effect of psychosocial factors is not always established they can play a crucial role in influencing direct risk markers as life events. Previous studies also suggest that both coping responses to stress may be influenced by temperamental traits that can influence the association between stress and mood episodes (Compas, Conner-Smith & Jaser, 2010). To add to the complexity, psychosocial factors like coping and temperament are also influenced by genetic factors themselves (Plomin & Crabbe, 2000).
Regarding the study to genetic factors, it is apparent that in the great majority of cases, genetic effects will involve multiple susceptibility genes where each of which only has a small effect (as also discussed in chapter 5). While it is still unclear whether all or even most of these genes will ever be identified, there will still remain an anonymous background genetic effects to be taken into account (Rutter, Pickles, Murray & Eaves 2001).
The complexity of these interactions has created the need for a more comprehensive methodology. Epigenetics, the study of changes in gene function that are mitoticalle and/or meiotically heritable and that do not entail a change in DNA sequence (Wu & Morris, 2001), is the next step in understanding the interactions of these factors. Epigenetic change is a relatively new frontier of temporally dynamic, reversible, molecular change that can be measured genome wide, revealing mechanisms of genomic control as well as consequences of environmental exposures. Epigenetic influences on gene regulation help mediate response and adaptation to the environment, accounting for part of the liability to psychiatric diseases (Schuebel, Gitik, Katharina & Goldman, 2016). Epigenetics also plays an important role when studying offspring

of parents with psychiatric illnesses. Although studies are limited, there is some evidence that different types of environmental stimuli can alter the epigenome of the whole brain or related neural circuits, contributing to long-lasting behavioural phenotypes that may be transmitted from parent to offspring via transgenerational mechanisms (Schuebel, et al., 2016; Post, 2016). Recent evidence points to a potentially transgenerational transmissible effect of stress on an epigenetic level, as holocaust exposure had an effect of FKBP5 methylanation that was observed in exposed parents as well as in their offspring (Yehuda et al., 2016).
While epigenetic studies of mental illness remain at early stages, understanding how environmental factors recruit the epigenetic machinery within specific brain regions to cause lasting changes in disease susceptibility and pathophysiology is revealing new insight into the aetiology and treatment of these conditions are an important next step.

CLINICAL IMPLICATIONS

This study provides insights that may become relevant in day-to-day clinical practice for the treatment and guidance and psychoeducation of patients and their social support system. Findings of **chapter 2 and 3** indicate that life events and psychological aspects play a role in the susceptibility for the onset and course of mood episodes and admissions. Therefore, they may be taken into account in the development of (early) intervention strategies. For individuals at risk for BD, structure in life and avoiding highly stressful situations may attribute to the prevention of illness onset and/or recurrence. For instance, training focusing on adopting active coping strategies to harness individuals against the effects of severe stressful events might be of additional value.
To date, studies to psychotherapeutic treatment with a focus on coping mechanisms and problem solving skills are limited.
In a randomized trial, Miklowitz et al. (2013) studied the effectiveness of a 4 month family focussed therapy among 40 youth at risk for mood disorders. Families followed sessions of family focused therapy, including psycho-education and training in social and problem solving skills or educational control sessions. Individuals of the treatment condition reported a sooner recovery of mood symptoms, a more favourable trajectory and more weeks in remission over 1 year of follow up.
In a more recent study by MacPherson et al. (2016), they evaluated mediators (family functioning, parent/child coping) on primary treatment outcome (child's mood and functioning) in a randomized trial of Child- and Familiy-Focused Cognitive Behavioral Therapy (CFF-CBT) versus Treatment As Usual (TAU) for pediatric BD. Several parent- and family factors significantly improved, specifically parenting skills and coping showed promise as mediators of child mood symptoms and global functioning. Although mediating effects for youth coping were not significant, this study highlights the importance of psychosocial factors in the early treatment of BD.
Taken together, the discussed studies show promising results for therapeutic interventions focussing on coping and problem solving skills, but more studies with larger samples and longer follow-up are required.

DIRECTIONS FOR FUTURE RESEARCH

We conducted an explorative study where we considered a few possible risk factors to be of influence in illness onset and course. Although the word 'risk factor' implies causality, this term includes both causal and predictive factors. Twin studies and offspring studies provide the design to further disentangle these factors. However, given the limitation in our studies of a small sample size, prospective longitudinal studies with adequate sample sizes are needed. Therefore, large national as well as international collaborations, such as ENIGMA, offer a great opportunity and a huge step forward in psychiatric research as they create enormous amounts of valuable data collected with harmonized measurement protocols. The ENIGMA Network consists of over 20 working groups worldwide that are collaborating on imaging genomics to understand brain structure, function, and disease based on brain imaging and genetic data (http://enigma.ini.usc.edu). In 2012, ENIGMA formed working groups on schizophrenia (van Erp et al., 2015), BD (Hibar and the CHARGE and ENIGMA2 Consortia, submitted for publication: Hibar et al, 2015a,b in press), major depression (Schmaal et al., 2015), and ADHD (Hoogman et al., 2015). Additional groups meta-analysing data on eight additional disorders have been formed since (Thompson et al., 2016). The initial goal of ENIGMA's disease working groups has been to meta-analyse effects of these disorders on the subcortical brain measures studies in the GWAS study. However, ENIGMA does not have the ideal design for all types of research. For the study to the impact of life events, or treatment outcomes for example, longitudinal designs instead of the cross-sectional comparisons in ENIGMA are preferred. International collaborations between longitudinal studies, like the BIOS and DBOS (Mesman, 2016) should be encouraged and supported as together they can create the power to solve (statistical) issues that the we are currently facing. In addition, prospective longitudinal studies should aim to limit the time between measurements to reduce time-bias in measurements and resolve the limitations that now prevent us from answering questions regarding causality.

A recent development which offers opportunities for not only research, but also prevention and treatment is the use of mobile E-health applications (Nicolas, Larsen, Proudfoot & Christensen, 2015; Faurholt-Jepsen et al., 2015). The use of smartphones in data collections allows respondents to monitor their mood state, levels of experienced stress and life events in real-time. Smartphones are not only a valuable tool in research, but can also be used to deliver interventions and psychoeducation, supplement treatment and enhance therapeutic reach in BD. Especially in adolescents, who are often hard to motivate to engage in face-to-face preventive therapy, offering an online tool or smartphone application could increase their engagement in (preventive) interventions (Faurholt-Jepsen et al., 2015). Although the evidence-based development of smartphone applications with the specific use for research or treatment is still in its infancy, it is a feasible and promising tool for an at risk population.

A valuable addition to life event measures, would be to combine the clinical interview data with biological measures that are known as biomarkers for the stress response (e.g. cortisol levels, ACTH levels, blood pressure). This would provide better insights in the direct physiologic effects of life stress especially if this could be com-

bined with brain measures as we established that hippocampal volume is influenced by severe life stress as well in **chapter 4**.
Inclusion of euthymic patients is often preferred in research in BD to minimize the bias in measurement. As we discussed in **chapter 5**, this could be providing a limited view on the disorder especially with measurements prone to change based on mood state, like inflammation. Therefore, measurements during both euthymic and mood episodes should be considered, as it might provide new insights on factors affecting the long-term course of the illness.

CONCLUDING REMARKS

In this thesis we described results of studies in two longitudinal research populations; the Dutch Bipolar Offspring Study and the Dutch Bipolar Twin Study. The general aim of these studies was to expand our knowledge on the role of life events as potential risk factor in the onset and course of BD.
The main finding is that severe life events influence the onset and recurrence of both mood episodes and admissions. In addition, psychosocial factors (passive coping style, harm avoidant temperament) proved to have a confounding effect on the influence of life events. These findings broaden our understanding on the role of life stress in BD and provide potential targets for (early) intervention.

CHAPTER 7

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CHAPTER 8

Nederlandse samenvatting
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NEDERLANDSE SAMENVATTING

Summary in Dutch

BIPOLAIRE STOORNIS

In Nederland wordt bij ongeveer 2% van de bevolking een bipolaire stoornis (ook wel bekend als manisch-depressieve stoornis) gediagnosticeerd. De bipolaire stoornis is een aandoening die wordt gekenmerkt door afwisselende periodes van overactiviteit en verhoogde (manische) stemming en periodes van verminderde activiteit met verlaagde (depressieve) stemming. Deze periodes van stemmingsontregeling kunnen enkele dagen tot vele maanden duren en worden afgewisseld met periodes van normaal functioneren. Vaak wordt de diagnose bipolaire stoornis als gevolg van geleidelijke ontwikkeling en niet onderkennen van de klachten, pas na jaren gesteld. In de DSM-IV (American Psychiatric Association, 1994) worden de bipolaire stoornissen verder gedifferentieerd in bipolaire stoornis I (depressie en manie), bipolaire stoornis II (depressie en hypomanie), cyclothyme stoornis (subsyndromale stemmingswisselingen) en de bipolaire stoornis niet anderszins gespecificeerd.
Duidelijk is dat de bipolaire stoornis vaker voorkomt in families, hetgeen een aanwijzing is dat genen een rol spelen bij de ontwikkeling van de stoornis. Zoals bij de meeste psychiatrische aandoeningen, gaat het hier om een interactie tussen genen en omgeving. Welke factoren daarnaast een rol spelen in de ontwikkeling van deze aandoening is vooralsnog een voortdurende zoektocht. In dit proefschrift worden in vier studies mogelijke oorzakelijke factoren zoals stress, afwijkingen in het volume van de hippocampus en activatie van het immuunsysteem en hun interacties met elkaar, onderzocht.

LEVENSGEBEURTENISSEN

Stress is een van de factoren die vaak in verband wordt gebracht met het ontstaan van psychiatrische stoornissen, zo ook met de bipolaire stoornis. Een van de manieren om stress te meten is door het in kaart brengen van doorgemaakte (stressvolle) levensgebeurtenissen.
Levensgebeurtenissen kunnen variëren van de dagelijkse beslommeringen (zoals een trein die vertraging heeft of een drukke dag op het werk) tot extreme situaties waar iemand zelf geen invloed op uit kan oefenen (zoals natuurrampen). Daarnaast kunnen we een onderscheid maken tussen positieve (i.e. afstuderen, nieuwe baan) en negatieve (i.e. ontslagen worden) levensgebeurtenissen en gebeurtenissen die wij zelf veroorzaken (i.e. kopen van een huis) of gebeurtenissen die ons overkomen (i.e. inbraak). Met al deze type gebeurtenissen is het goed voor te stellen dat het in kaart brengen van levensgebeurtenissen en de mogelijk ervaring van stress die daarop volgt een uitdaging is.
De methode die in dit proefschrift gehanteerd wordt om levensgebeurtenissen in kaart te brengen, is de Life Events and Difficulties Schedule (LEDS) van Brown & Harris. De LEDS is een semigestructureerd interview waarbij er gedetailleerde informatie wordt verzameld over de betreffende gebeurtenis, wanneer het gebeurde en de context waarin het gebeurde. Middels 10 categorieën worden de gebeurtenissen in kaart gebracht, te weten; opleiding, werk, voortplanting, wonen, geld/bezittingen,

misdaad/wet, gezondheid, relaties/partners, andere relaties en overige/overlijden. Na het interview wordt hier een verslag over geschreven door de interviewer. Vervolgens gaan twee onafhankelijke beoordelaars elke genoemde gebeurtenis uit het verslag een 'ernst-score' geven. Deze ernst-score varieert tussen 1 (milde gebeurtenis) en 4 (zeer ernstige gebeurtenis) en is afhankelijk van de 'contextuele ernst', oftewel; 'Wat de meeste mensen zouden ervaren bij een gebeurtenis onder bepaalde omstandigheden'. Omdat de beoordelaars niet bij het interview zijn geweest, zijn zij blind voor de emoties en reactie van de persoon tijdens het omschrijven van de gebeurtenis en hebben zij enkel de informatie over de omstandigheden van de gebeurtenis. Zij kunnen hierdoor een onafhankelijk oordeel geven over de score van de ernst. De totale scores hiervan (per jaar of totaal) geven uiteindelijk weer in welke mate iemand stress heeft ervaren.

DE STUDIES

De studies die worden omschreven in dit proefschrift maken gebruik van data verkregen uit de Kinderen Bipolaire Ouders studie (KBO) en de Bipolaire Tweelingen studie (BTS). Hieronder zal ik beide studies kort omschrijven.

KBO-project

In hoofdstuk 3 wordt een studie uit het KBO-project beschreven. Het KBO-project is een prospectieve Nederlandse cohortstudie naar de ontwikkeling van kinderen van een ouder met een bipolaire stoornis. Omdat de bipolaire stoornis vaker voorkomt in families, zijn kinderen van een ouder met een bipolaire stoornis een interessante doelgroep voor onderzoek naar het vroege beloop van de bipolaire stoornis en eventuele beschermende- en risicofactoren die hier een rol in spelen. Tussen 1997 en 2011 werd een groep van 140 kinderen uit 86 families opgevolgd. De deelnemers waren bij aanvang van de studie gemiddeld 16 jaar (variërend van 12 tot 21 jaar) en waren bij de meest recente meting gemiddeld 28 jaar. De deelnemers werden in de afgelopen jaren viermaal uitgebreid onderzocht: bij aanvang van de studie, na 1 jaar, na 5 jaar en na 12 jaar. De bevindingen van al deze metingen zijn beschreven in vier eerder verschenen proefschriften (Wals, 2004; Reichart, 2005; Hillegers, 2007; Mesman, 2015) .

Bipolaire tweelingen studie

In de hoofdstukken 2, 4 en 5 worden studies omschreven uit een Nederlandse longitudinale tweelingstudie. Hierbij werden tweelingen, zowel een- als twee-eiig waarvan een of beide een bipolaire stoornis hebben, gemeten op twee verschillende tijdstippen. Daarnaast werd ook een controlegroep met gezonde tweelingen geïncludeerd. De eerste meting vond plaats tussen 2001 en 2006 en de tweede tussen 2009 en 2011. De tweelingen waren tussen de 18 en 60 jaar oud. In drie eerder verschenen proefschriften worden de bevindingen van deze studie omschreven (Van der Schot, 2009; Vonk, 2015; Bootsman, 2016).

Het doel van een tweelingstudie is om de relatieve bijdrage van genen en omgevingsfactoren aan een bepaalde eigenschap te schatten. Dit is mogelijk door te kijken naar de verschillen tussen eeneiige en twee-eiige tweelingen. Eeneiige tweelingen delen 100% van hun genen en twee-eiige tweelingen ongeveer 50% (gelijk aan dat

van andere broers en zussen). Met dit gegeven kan vervolgens gerekend worden. Wanneer een bepaalde eigenschap in hoge mate erfelijk is, dan moet er in eeneiige tweelingen dus in hogere mate overeenkomsten worden gevonden dan in twee-eiige tweelingen.

HOOFDSTUK 2

Hoofdstuk 2 richt zich op de rol van levensgebeurtenissen en in welke mate zij bijdragen aan de kans op een eerste en eventuele daaropvolgende psychiatrische opnames bij patiënten met een bipolaire stoornis. Wij vonden aanwijzingen dat het aantal en de ernst van levensgebeurtenissen welke iemand meemaakt invloed hebben op een eerste en, in mindere mate, op een daaropvolgende psychiatrische opname. Daarnaast bleek ook het aantal opnames in het verleden een voorspellende factor te zijn op de kans op een nieuwe opname. Hoe hoger het aantal opnames in het verleden, hoe hoger de kans dat er een nieuwe opname plaatsvindt. Dit ondersteunt eerdere studies waaruit is gebleken dat het risico op een herhaalde opname hoog is, te weten tussen de 50 -75% van de patiënten heeft een tweede opname binnen 4 tot 5 jaar na een eerste opname.
Een invloedrijke theorie bij onderzoek naar stemmingsstoornissen is de 'kindling hypothese'. Deze theorie veronderstelt dat levensgebeurtenissen/stress vooral een rol spelen bij de eerste stemmingsepisode(s) en dat latere daaropvolgende episodes daar meer en meer los van komen te staan. Deze theorie is in deze studie getoetst bij de herhaalde psychiatrische opnames. Hoewel de invloed van levensgebeurtenissen op de kans op een opname af nam naarmate het aantal opnames toenam, vonden wij geen overtuigend bewijs voor de hierboven genoemde 'kindling hypothese'.

HOOFDSTUK 3

In hoofdstuk 3 wordt een studie omschreven die zich richt op de rol van levensgebeurtenissen bij het ontstaan van stemmingsstoornissen bij kinderen van een ouder met een bipolaire stoornis. De deelnemers werden op alle vier de metingen uitgebreid ondervraagd aan de hand van de LEDS. Net als bij de eerste meting van de Nederlandse KBO-studie (Hillegers et al., 2005) vonden we ook na 12-jaar follow-up op de vierde meting aanwijzingen voor een relatie tussen stressvolle levensgebeurtenissen en het ontstaan van een eerste stemmingsepisode. Het risico op een recidiverende stemmingsstoornis is hoog, te weten 33%. Om deze reden werd in dit hoofdstuk ook onderzocht of levensgebeurtenissen ook een rol spelen in het beloop van deze stoornis. Hieruit blijkt dat levensgebeurtenissen ook bij een recidief een rol spelen. Echter, het aantal stemmingsepisodes in de voorgeschiedenis is ook een sterke voorspeller voor het optreden van een recidief. Dit suggereert dat er mogelijk sprake is van een 'kindling' effect, zoals al eerder in hoofdstuk 2 werd besproken, maar ook hier vonden we voor dit effect geen overtuigend bewijs.
Ook persoonlijkheidsaspecten lijken een rol te spelen bij de kwetsbaarheid van stemmingsstoornissen. Over hoe deze de relatie tussen levensgebeurtenissen en stemmingsstoornissen beïnvloeden is echter weinig over bekend. In een vervolgstap werd daarom gekeken naar een aantal persoonlijkheidsaspecten, zoals de manier waarop omgegaan wordt met problemen (coping), temperament en de manier hoe kinderen de opvoedingsstijlen van hun ouders ervaren. Hieruit blijkt een associatie

te bestaan tussen passieve coping (een ontwijkende, afwachtende houding bij probleemsituaties) en stemmingsepisodes. Opmerkelijk is dat het toevoegen van passieve coping aan het statistische model van invloed is op de sterkte van de associatie tussen levensgebeurtenissen en stemmingsstoornissen, een zogenaamd 'confounding' effect. Bij een eerste stemmingsepisode, lijkt de relatie tussen levensgebeurtenissen en stemmingsgevoeligheid te versterken, bij recidiverende stemmingsstoornissen doet passieve coping het effect van levensgebeurtenissen echter vervagen. Dit laatste geldt ook voor een conflict vermijdend temperament en recidiverende stemmingsstoornissen. Deze resultaten suggereren dat niet enkel stressvolle levensgebeurtenissen, maar ook psychologische-/persoonlijkheidsaspecten een belangrijke rol spelen bij de kwetsbaarheid voor (recidiverende) stemmingsstoornissen en zijn dus een mogelijk aangrijpingspunt voor vroeg interventie.

HOOFDSTUK 4

In hoofdstuk 4 is een studie beschreven die is uitgevoerd onder gezonde tweelingen. Het doel van deze studie was om te analyseren in hoeverre het volume van de hippocampus – een zeer belangrijk en prominent hersengebied dat betrokken is bij stressregulatie en geheugen, en welke diep in de hersenen ligt – samenhangt met levensgebeurtenissen. Een van de onderliggende hypothesen was dat stressvolle levensgebeurtenissen de hippocampus negatief beïnvloeden waardoor mogelijk het vermogen van de hippocampus tot het reguleren van stress beperkt wordt. Hiervoor hebben we gekeken naar het volume van de hippocampus en niet zozeer zijn functie. Uit de resultaten bleek dat met name zeer ingrijpende levensgebeurtenissen negatief samenhangen met volume van de hippocampus. Dat wil zeggen dat de groep mensen in deze studie relatief meer stressvolle levensgebeurtenissen hadden meegemaakt een kleiner volume van de hippocampus lieten zien. Verder bleek dat het vooral de stressvolle levensgebeurtenissen zijn die gedeeld werden binnen tweelingparen samenhingen met een kleiner volume van de hippocampus en niet zozeer gebeurtenissen die uniek waren voor het individu. Wel is er nog meer onderzoek nodig om dit te bevestigen, zeker aangezien de groep deelnemers aan de studie relatief klein was.

HOOFDSTUK 5

In hoofdstuk 5 omschrijven we een studie welke een vervolg is op een eerdere studie uit onze groep over de relatie tussen de bipolaire stoornis en het immuunsysteem. In deze eerdere studie werden aanwijzingen gevonden voor een relatie tussen pro-inflammatoire monocyten (cellen die ontstekingsreacties bevorderen) en de bipolaire stoornis welke vooral verklaard werd door gedeelde omgevingsfactoren. Uit eerdere studies is gebleken dat stress een mogelijk omgevingsfactor is die invloed kan uitoefenen op zowel het immuunsysteem als de bipolaire stoornis. In onze studie hebben wij gekeken of deze gedeelde omgevingsfactoren verklaard zouden kunnen worden door levensgebeurtenissen, maar hier zijn geen aanwijzingen voor gevonden. De immunologie is een onderzoeksveld dat voortdurend in ontwikkeling met vele nieuwe inzichten in het psychiatrische veld. Zo zou de staat van de pro-inflammatoire monocyten mogelijk ook onder invloed staan van de stemmingsepisode waar de patiënten zich in bevinden. Er is meer onderzoek nodig om deze relatie beter te begrijpen.

DISCUSSIE & CONCLUSIE

Het doel van dit proefschrift was om meer kennis te vergaren over de rol die levensgebeurtenissen spelen als potentiele risicofactor op het ontstaan en het beloop van de bipolaire stoornis.

In hoofdstuk 6 worden de studies, zoals omschreven in dit proefschrift, samengevat. Tevens worden er enkele methodologische afwegingen van de studie besproken, zoals onder andere de uitdagingen die het tweelingen onderzoek met zich meebrengt en de generaliseerbaarheid van de studies. Daarnaast wordt er stilgestaan bij de klinische implicaties en wordt er gespeculeerd over hoe de toekomst van onderzoek naar risico factoren en de relatie met het ontstaan en beloop van psychiatrische stoornissen eruit zou moeten zien.

Concluderend, is de belangrijkste bevinding van dit onderzoek de invloed welke stressvolle levensgebeurtenissen op het ontstaan en beloop van de bipolaire stoornis uitoefenen. Wij vonden aanwijzingen dat stressvolle levensgebeurtenissen invloed uitoefenen op zowel het ontstaan van stemmingsepisodes als op een verhoogd risico op een psychiatrische opname. De invloed hiervan was groter op de eerste stemmingsepisode of opname dan op eventueel daaropvolgende episodes of opnamen. Bovendien bleken psychologische-/persoonlijkheidsaspecten, zoals coping strategieën, te interacteren met de invloed die levensgebeurtenissen hebben. Hoewel er meer onderzoek nodig is, dragen bovenstaande bevindingen bij aan onze kennis van de rol van stress bij de bipolaire stoornissen en biedt het mogelijke handvatten voor (vroege) interventie.

LIST OF PUBLICATIONS

PEER-REVIEWED PUBLICATIONS

Kemner, S.M., van Haren, N.E.M., Bootsman, F., Eijkemans, M.J.C., Vonk, R., van der Schot, A.C., Nolen, W.A. & Hillegers, M.H.J (2015). The influence of life events on first and recurrent admissions in bipolar disorder. International Journal of Bipolar Disorders, doi:10.1186/s40345-015-022-4.

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Bootsman, F., **Kemner, S.M.**, Hillegers, M.H.J., Brouwer, R.M., Vonk, R., van der Schot, A.C., Hulshoff Pol, H.E., Nolen, W.A., Kahn, R.S. & van Haren, N.E.M. (2016). The association between hippocampal volume and life events in healthy twins. Hippocampus, 26(8), 1088-95.

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Bootsman, F., Brouwer, R.M., Schnack, H.G., **Kemner, S.M.**, Hillegers, M.H.J., Sarkisyan, G., van der Schot, A.C., Vonk, R., Hulshoff Pol, H.E., Nolen, W.A., Kahn, R.S & van Haren, N.E.M. (2016). A study of genetic and environmental contributions to structural brain changes over time in twins concordant and discordant for bipolar disorder. Journal of Psychiatric Research, 79, 116-24.

MANUSCRIPTS IN PROGRESS

Kemner, S.M., Brouwer, R.M., Haren, N.E.M., de Wit, H.J., Kahn, R.S., Nolen, W.A., Drexhage, H.A. & Hillegers M.H.J. (submitted). Is stress the common environmental factor explaining the relationship between pro-inflammatory monocytes and bipolar disorder? Results from the Dutch Bipolar Twin Study.

DANKWOORD

Klaar, af, finite, basta! 5,5 jaar na mijn start als promovenda is het dan eindelijk zover. Terwijl ik dit typ kan ik zelf nog nauwlijks geloven dat het (bijna, want de verdediging komt nog) is afgelopen. Met name de afgelopen 3 jaar was het niet altijd makkelijk om gemotiveerd te blijven en mijn vrije uren toe te wijden aan het afronden van dit proefschrift. Zonder de motivatie, steun en vertrouwen van een groot aantal mensen was het afronden absoluut niet mogelijk geweest.
Ten eerste wil ik alle deelnemers aan zowel de DBTS (tweelingstudie) als de DBOS (KBO-studie) hartelijk bedanken. Zonder jullie deelname en inzet was dit proefschrift (en een groot aantal eerder verschenen proefschriften) niet mogelijk geweest.

Willem Nolen, promotor. Beste Willem, ik kan mij nog heel goed een moment herinneren waarop ik het even niet zag zitten. Ik wist niet of het nog nut had om door te gaan. Jij zei; "We gaan er nog een jaar voor, als je er over een jaar nog zo voorstaat dan kunnen we denken aan stoppen". Een jaar later waren er 2 artikelen gepubliceerd en nog een jaar later werd mijn proefschrift goedgekeurd. Jouw nuchtere kijk op zaken maakte dat ik er zelf ook vertrouwen in kreeg. Daarnaast zorgde je altijd voor snelle feedback. Ik ben enorm blij dat jij mijn promotor bent. Enorm bedankt voor alles!

René Kahn, promotor. Helaas kan u niet aanwezig zijn tijdens mijn verdediging, vanwege een hele mooie stap die u heeft gemaakt naar het Mount Sinai ziekenhuis in New York. Bedankt voor de altijd kritische input op de artikelen. Ik wens u heel veel succes in The Big Apple!

Manon Hillegers & **Neeltje van Haren**, co promotoren. Beste Manon en Neeltje, ik vond dat ik altijd een luxepositie had met twee co-promoteren met verschillende kennisvelden in de buurt. Ik kon altijd aankloppen met vragen. Zelfs na mijn vertrek uit het UMC Utrecht wisten jullie altijd tijd voor mij te maken (zelfs 's avonds of in het weekend) en mij snel van input te voorzien wanneer nodig. Jullie zijn altijd positief gebleven en wisten mij te motiveren. Dank voor de fijne samenwerking!

René Eijkemans & **Rachel Brouwer,** mijn statistische hulplijnen. Hartelijk dank voor jullie hulp en bijdrage aan de studies en altijd geduldige uitleg.

Geachte leden van de leescommissie, **Prof. Dr Hulshoff Pol, Prof. Dr. Kupka, Prof. Dr. Ormel,** hartelijk dank voor jullie beoordeling van het manuscript.

Ronald Vonk en **Astrid van der Schot**, zonder jullie werk en inzet op de eerste meting van de tweelingstudie was mijn proefschift niet mogelijk geweest. Dank! Daarbij is natuurlijk ook het lab in Rotterdam cruciaal geweest: **Hemmo** (bedankt voor de hulp en skype sessies vanuit Ecuador), **Harm, Angelique, Annemarie**. Dank voor alle hulp rondom de immuno data!

Mijn paranimfen, Esther en Annabel. Lieve **Esther**, vriendin en paranimf, zonder jou was dit allemaal niet mogelijk geweest. Toen ik als stagiaire nog groen als gras het onderzoek van de cannabis quest inrolde hadden we direct een klik. Wij wisten beide wel hoe we de dingen moesten regelen en de touwtjes in handen konden houden. Vanaf het eerste moment had jij het volle vertrouwen in mij. Ik ben enorm blij dat wij al die tijd zo nauw samen hebben mogen werken en dat we zelfs na het verlaten van het UMC Utrecht goed contact hebben gehouden. Van bootcamp tot borrel, het is allemaal even gezellig! Bedankt voor alles! **Annabel**, jouw oneindige enthousiasme voor de wetenschap en enorme doorzettingsvermogen werken aanstekelijk. Vanaf onze eerste echte kennismaking, ons eerste tripje naar Istanbul, heb ik altijd verschrikkelijk met je kunnen lachen. Jouw open- en eerlijkheid zijn zeldzaam en een fantastische eigenschap.

Florian, samen met Esther vormden wij een geoliede machine. Wat moet jij ons af en toe met met argwaan hebben bekeken als wij meer tijd kwijt waren aan het bespreken van de nieuwste ontwikkelingen omtrent Sylvie en Rafael dan aan het doen van analyses, maar het is ons nu alle drie gelukt! Ik heb veel van je mogen leren. Dank voor alles!

De 5BL – **Esther, Lucija, Annabel & Sanne**. Wat was het heerlijk om tijdens onze etentjes mijn hart te luchten en ervaringen te delen. Laten we ook de fantastische tijd in Istanbul (And now welcoming to the stage…) en Miami (fietsen & moijito's is een super goede combi) niet vergeten. Dat er nog maar veel etentjes mogen volgen!

En alle anderen die nauw betrokken waren bij het traject. De 'lotgenoten' van kantoortuin 1; **Marloes, Sven, Marc, Thalia, Janna Marie, Marriet, Bram**. Alle stagiaires die meewerkten aan de KBO studie en aan de Bipolaire Tweelingen studie: **Nina, Mylene, Anneloes, Roos, Hannah, Farkhonda, Tamara, Anne, Hans, Afanaisa en Nanja**. Bedankt voor jullie inzet en harde werk!

Dan is er ook nog een grote groep mensen die buiten het UMC Utrecht en het promoveren impliciet hebben bijgedragen aan dit proefschrift. Met name door mij mentaal te ondersteunen en te zorgen dat ik ook de nodige ontspanning opzocht.

Maaike & **Gerlinde**; bedankt voor de onvoorwaardelijke steun en begrip als ik weer eens vroeg naar huis ging tijdens een borrel, of de borrel in zijn geheel moest overslaan om de volgende ochtend weer fris en fruitig in de OBA aan de slag te kunnen. Jullie zijn toppers!

Al mijn andere lieve vrienden, bedankt voor de hoognodige momenten van gezelligheid en ontspanning; **Charissa, Marloes, Mirjam, Jennie, Laura, Maaike, Jorrit, Koos**. De dansmeisjes (en de mannen), **Kathinka, Hannah, Debora** en **Nancy**. Dank voor jullie positiviteit en gezelligheid. En alle andere lieve mensen om mij heen. Ik ben een gezegend mens dat de namen die ik hier noem nog lang niet alle mensen zijn die het leven een feestje (heel vaak letterlijk!) maken – jullie weten wie jullie zijn!

Lieve familie en schoonfamilie: **Paps & Helen**, **Nina & Ben**, **Ko & Marian**, **Alex & Marisa**. Hoewel het voor jullie misschien niet altijd even duidelijk was waar ik mee bezig was waren jullie altijd geïnteresseerd hoe het er voorstond, dank jullie wel voor jullie steun!

Lieve **mams**, bedankt voor alles (ja, alles!).

Douwe Hemrika, niet meer onder ons, maar nog altijd een bron van inspiratie.

In de afgelopen tweeeneenhalf jaar combineerde ik het onderzoek met een functie buiten de wetenschap, eerst als marketing coordinator en later als manager van het marketing team bij Bannerconnect, een bedrijf gespecialiseerd in online advertising technologie. Hoewel dit niet altijd een gemakkelijke combinatie is geweest en promoveren hiermee een uit de hand gelopen hobby leek heb ik het als een waardevolle combinatie van twee hele verschillende werelden beschouwd. Het combineren was niet mogelijk geweest zonder de steun van een aantal bijzondere collega's aldaar. **Rutger Tuit**, 'ik ben bijna klaar' werd 'het duurt nog drie jaar'. Dank voor je begrip, vrijheid en ruimte. Zonder deze baan waar ik zoveel energie uit haal was het me misschien nooit gelukt. Mijn dank is groot! Hopelijk kunnen we in de toekomst nog eens samenwerken! **Geri, Nina en Deesi** en al mijn andere (ex-) **Bannerconnect collega's**, werken met jullie was en is een groot feest!

And special thanks to **Francisco Paramos**. My first hire, who made an amazing first impression because of a design assignment that is now the cover of this thesis. Thank you for letting me use this picture and designing my cover!

Last, but certainly not least. Lieve **Rick**, bedankt voor alle lieve zorgen, alle ruimte die je mij hebt gegeven, het geduld en vertrouwen. Toen ik jou leerde kennen was ik net begonnen met mijn promotietraject en had ik nog geen idee wat mij (en ons) te wachten stond. Jij hebt mij door het hele proces bijgestaan, alle ups- en downs, jij was er voor mij. Ik kan in woorden niet uitdrukken hoe dankbaar en gelukkig ik ben met een man als jou aan mijn zijde.

CURRICULUM VITAE

Sanne Mariska Kemner was born in Hoorn in the Netherlands on November 9th 1984. She studied Psychology at the Free University in Amsterdam from September 2003 until February 2008. In February 2008, she completed her Master's degree Cum Laude in Clinical Neuropsychology. After obtaining her Master's degree she continued her study in a second Masters in Clinical Psychology. During her second Master's she worked parttime as a neuropsychologist at GGZinGeest in the 'Valeriuskliniek' in Amsterdam from June to October in 2009. During the same period she started a research internship at the CannabisQuest, a large epidemiological study focusing on the association of cannabis use and psychotic symptoms, at the University Medical Center Utrecht (UMCU). After the internship, she started working as a research assistant for the Dutch Bipolar Offspring Study (DBOS) and the Dutch Bipolar Twin Study (DBTS) in the UMCU in November 2009. In August of 2011, she started her PhD-Candidacy at the UMCU, studying the impact of life events on bipolar disorder in the above-mentioned studies (DBOS & DBTS). Her PhD-candidacy was supervised by Manon H.J. Hillegers, MD, PhD, Neeltje E.M. van Haren, PhD, René S. Kahn, MD, PhD and Willem A. Nolen, MD, PhD. In February 2014, she stared a career outside of academics as a marketing coordinator at Bannerconnect, a service and technology provider for online marketing solutions. She is currently working at Bannerconnect as marketing manager and member of the management team.